

## OCCURRENCE OF *HELICOBACTER* SPECIES WITH SPECIAL PRIORITY TO *H. PYLORI* IN HEN'S EGGS

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**Received:** 24 June 2018; **Accepted:** 9 July 2018

### ABSTRACT

A total of 300 random eggs, representing 60 samples, (30 from baladi hens, and 30 from poultry farms) were collected from different markets, poultry farms, groceries and supermarkets located in Assiut governorate, Egypt. Each egg sample composed of 5 eggs and these samples were examined for the incidence of *Helicobacter* species using *Helicobacter pylori* special peptone agar (HPSPA). The results revealed that the incidence of *Helicobacter* species from examined baladi hen's egg shells was 10 (33.33%), poultry farms hen's egg shells was 9 (30%) and 1 (3.33%) from examined baladi hen's egg content samples. *H. pylori* was isolated in a percentage of 5 (16.67%) from examined baladi hen's egg shells while from poultry farms hen's egg shells was 4 (13.33%). In addition, other *Helicobacter* species were isolated from the examined samples as *H. cinaedi* in a percentage of (6.67%) from examined baladi hen's egg shells and (10%) from examined poultry farms hen's egg shells. *H. felis* was isolated in a percentage of (6.67%) from both examined baladi and poultry farms hen's egg shells samples and *H. pullorum* was isolated in a percentage of (3.33%) from examined baladi hen's egg shells. Also, it was isolated from examined baladi hen's egg contents in a percentage of (3.33%). Identification of ureC gene for *H. pylori* isolated from both baladi hen's egg shells and poultry farms hen's egg shells by using polymerase chain reaction (PCR) revealed that 8 from 9 samples were positive for *H. pylori* by using (PCR) in a percentage of (88.89%).

**Key words:** *Helicobacter*, *H. Pylori*, Hen's Eggs.

### INTRODUCTION

Eggs are among the few foods that are used throughout the world regardless of religion and ethnic groups (Stadelman and Cotterill, 2001). The hen's egg is one of the perfectly preserved biological items found in nature and its nutritional benefits are well recognized. Eggs remain a stable food within the human diet, consumed by people throughout the world in various dishes and considered very nutritious and a cheap source of protein (Osei-Somuah *et al.*, 2003).

Eggs also have many functional properties such as foaming, emulsifying and unique color and flavor, which are important in several food products (Stadelman and Cotterill, 2001). Freshly laid eggs are generally devoid of organisms, however, following exposure to environmental conditions, may become

contaminated with different types of microorganisms (Ellen *et al.*, 2000). Eggs are liable to contamination either before laying (congenitally) or after laying (extragenitally), when the microorganisms reach the egg contents through penetration pores of shell cause low egg quality, low shelf life, low safety and induce public health hazards (Board and Fuller, 1994). In addition, fecal matter, improper washing, using of contaminated water and bad handling are the common sources of contamination. In spite of their high nutritive value, eggs were responsible for several outbreaks and were a vehicle for transmission of certain human pathogens as *Helicobacters* (Miyamota *et al.*, 1998; Hangombe *et al.*, 1999; Gast *et al.*, 2004).

*Helicobacter* is a genus of Gram-negative bacteria possessing a characteristic helical shape. They were initially considered to be members of the *Campylobacter* genus, but in 1989 Goodwin *et al.* published sufficient reasons to justify his new genus name of *Helicobacter*. The *Helicobacter* genus contains about 35 species and some species are pathogenic and known to colonize the gastrointestinal and biliary tracts of many animal species. They are

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grouped into two groups gastric and enterohepatic *Helicobacter* species (Boyanova, 2011).

Gastric *Helicobacter* species including *H. pylori*, *H. acinonychis*, *H. bizzozeronii*, *H. felis*, *H. mustelae*, *H. nemestrinae*, *H. salomonis* and *H. suncus*. Enterohepatic *Helicobacter* species as *H. pullorum*, *H. bilis*, *H. canadensis*, *H. canis*, *H. cholecystus*, *H. cinaedi*, *H. fennelliae*, *H. ganmani*, *H. hepaticus*, *H. mesocricetorum*, *H. muridarum*, *H. pametensis*, *H. rodentium*, *H. trogonium* and *H. typhlonicus* (Solnick and Vandamme, 2001).

There are many *Helicobacter* species that infect human and leading to many medical condition, as *H. helimannii* which leads to duodenal ulcer, gastric carcinoma and mucosa associated lymphoid tissue (MALT) tumors. Also *H. felis* which is usually associated with gastric disease and many species isolated from human diarrheal samples included *H. cinaedi*, *H. canis*, *H. pullorum*, *H. fennellae*, *H. canadensis* and *H. pylori*. Additionally, there are *Helicobacter* species have been isolated from livers and have been associated with hepatic diseases as *H. hepaticus* which lead to hepatitis and hepatic carcinoma (On *et al.*, 2002).

The best known and the most important in terms of global impact on human disease is *H. pylori* in which the definitive reservoir is assumed to be humans (Drumm *et al.*, 1990). The history of discovering and isolation of *H. pylori* was first documented since a century ago. *H. pylori* was first discovered in the stomachs of patients with gastritis and ulcers in 1982 by (Marshall and Warren 1983) and they awarded the 2005 Nobel Prize in Physiology or Medicine. Acute infection with *H. pylori* may appear as an acute gastritis with abdominal pain or nausea (Butcher, 2003) which develop to chronic gastritis. The symptoms are often stomach pains, nausea, bloating, belching, and sometimes vomiting or black stool (Ryan, 2010).

About 10-20% of those infected with *H. pylori* develop gastric and duodenal ulcers. *H. pylori* infection is also associated with 1-2% lifetime risk of stomach cancer and less than 1% risk of gastric mucosa associated lymphoid tissue lymphoma (MALT) (Kusters *et al.*, 2006). *H. pylori* has been recognized as the principal cause of gastric disease which is more severe than that caused by *H. helimannii* and cause gastritis, peptic ulcer, gastric carcinomas, and mucosa-associated lymphoid tissue lymphoma (Komoto *et al.*, 1998). *H. pylori* was classified as a definitive carcinogen to human which may play a causative role in development of up to 90% of gastric cancers (Forman *et al.*, 1994).

There was association between *H. pylori* infection and hypertension and myocardial infarction. (Whincup *et*

*al.*, 1996). *H. pylori* infection presents approximately in half of the world's population (Lambert *et al.*, 1995). The organism can be found in 70-90% and 25-50% of the population in developing and developed countries, respectively (Sykora *et al.*, 2006; Vale and Vitor, 2010) depending on environmental and socioeconomic factors. In spite of the general idea about the low prevalence of gastric cancer, *H. pylori* infection considered the fourth most common type of cancer and the second leading cause of cancer-related deaths worldwide (WHO, 2010). The minimum infectious dose of *H. pylori* during primary and secondary infection was  $10^4$  bacteria (Solnick *et al.*, 2001).

Moreover, Enterohepatic *Helicobacter* species, including *H. pullorum*, is increasingly recognized as microbial pathogen in humans and animals (On *et al.*, 1996; On *et al.*, 2002). *Helicobacter pullorum* was first described by Stanley *et al.* (1994). On *et al.* (1996) indicated that *H. pullorum* was originally isolated from the feces, the intestines and damaged livers of broilers and laying hens (Burnens *et al.*, 1994; Stanley *et al.*, 1994), and also from the feces of humans (Ceelen *et al.*, 2005). *H. pullorum* has been related to enteritis and hepatitis in broiler chickens and laying hens, diarrhea, gastroenteritis, and liver disease in humans (Burnens *et al.*, 1994; Stanley *et al.*, 1994; Young *et al.*, 2000 and Ceelen *et al.*, 2005). The organism can be considered a food borne human pathogen (Ceelen *et al.*, 2006).

Fox *et al.* (1998) observed that *H. pullorum*, *H. bilis* and *H. rappini* were found to cause some kinds of extra digestive diseases in liver and gall bladder of human with chronic cholecystitis.

Since the isolation of *Helicobacter* species from eggs is very scarce and to determine whether eggs act as a vehicle in transmission of *Helicobacter* species, therefore this work was planned to study the incidence of *Helicobacter* species in hen's eggs, in addition to identification and confirmation of *H. pylori* ure C gene by using PCR.

## MATERIALS and METHODS

### Isolation and identification of *Helicobacter* species from hen's eggs.

#### Collection of samples:

A total of 300 random eggs, representing 60 samples, (30 from baladi hens', and 30 from poultry farms) were collected from, poultry farms, groceries and supermarkets located in Assiut Governorate, Egypt. Each egg sample (composed of 5 eggs) was placed in a sterile plastic bag and dispatched to the laboratory with a minimum of delay where they were prepared and examined.

**Preparation of samples:**

**Egg shells:** Egg shells were tested by a surface rinse method as described by Moats (1980).

**Egg contents:** The egg sample was prepared for evacuation of its content according to Speck (1976).

**Isolation of *Helicobacter* species:** The technique adopted by Stevenson *et al.* (2000) was used.

**Enrichment:** One milliliter of rinsed solution and egg content samples were aseptically inoculated into a sterile test tubes containing 10 ml of selective enrichment broth *Helicobacter pylori* special peptone broth (HPSPB) containing selective supplement which is Vancomycin 5.0 mg, Trimethoprim lactate 2.5 mg, Cefsulodin 2.5 mg and Amphotericin B 2.5 mg. The inoculated tubes were incubated at 37°C for 48 hours in an atmosphere of 6% O<sub>2</sub>, 10% CO<sub>2</sub> and 84% N<sub>2</sub> by CO<sub>2</sub> incubator (HERA cell 150 Thermo scientific).

**Selective plating:** Loopfulls from incubated broth cultures were then streaked on HPSPA supplemented with *Helicobacter* selective supplement (Oxoid Code SR147E). All the cultured plates were inspected after 3, 5 days. Suspected colonies grow, slowly, small not exceeding 2 mm in diameter, translucent, circular and convex.

**Identification of *Helicobacter* (Solnic and Vandamme, 2001):** Gram stain and Biochemical characteristics which include Oxidase production test, Catalase production test, Urease production test, Hippurate hydrolysis test, Nitrate reduction test, Glycine tolerance test and Salt tolerance test (Solnic and Vandamme, 2001).

**Identification of ureC gene for *H. pylori* by using PCR.****DNA Extraction using QIA amp kit (Shah *et al.*, 2009):**

After overnight culture on nutrient agar plates, one or two colonies were suspended in 20 ml of sterile distilled water, and the suspension was then heated at 100°C for 20 minutes. Accurately, 50-200 µl of the culture were placed in Eppendorf tube and Equal volume from the lysate (50-200 µl) was added, after addition of 20-50µl of proteinase K, the mixture was

incubated at 56 °C for 20-30 min. After incubation, 200 µl of 100% ethanol was added to the lysate. The solution was added to the column and centrifuged at 8000 rpm for 1 min, then the filtrate was discarded. The sediment was washed using AW1 buffer (200 µl), the column was centrifuged at 8000 rpm / 1 min, and the filtrate was discarded. Washing was applied by using the AW2 buffer (200µl), the column was centrifuged at 8000 rpm / 1 min, and the filtrate was discarded. The column was placed in a new clean tube then, 25-50 µl from the Elution buffer was added, centrifuged at 8000 rpm/1min, and then the column was discarded. The filtrate was put in clean tube containing the pure genomic DNA and nucleic acid was eluted with 100 µl of elution buffer provided in the kit.

**Amplification reactions of DNA:****Amplification of ureC gene for *H. pylori* (Kianpour *et al.*, 2014):**

The amplification was performed on a Thermal Cycler (Master cycler, Eppendorf, Hamburg, Germany) using 50 µL containing 5 µL 10 × buffer + MgCl<sub>2</sub>, 2 mM dNTP, 2 unit Taq DNA polymerase, 100 ng genomic DNA as a template, and 25 picomole of each primer. PCR was performed using a thermal cycler (Eppendorf Co., Germany) under the following conditions: an initial denaturation for 10 minutes at 94°C; 35 cycles for 1 minute at 94°C, 1 minute at 55°C, 1 minute at 72°C, and a final extension at 72°C for 10 minutes.

PCR amplified products were analyzed by 1.5% of agarose gel electrophoresis in 1x TBE buffer stained with ethidium bromide and visualized on UV transilluminator. A 100 bp plus DNA Ladder was used to determine the fragment sizes and the gene product was 294 bp.

**Primer sequences used for PCR identification system:**

Application of PCR for detection of urease C gene (ureC) aka phosphoglucosamine mutase gene (glmM) specific for identification and characterization of *H. pylori* was performed essentially by using Primers (Pharmacia Biotech) as shown in the following table:

Target gene	Oligonucleotide sequence (5' → 3')	Product size (bp)	References
ureC (F)	5' GAATAAGCTTTTAGGGGTGTTAGGGG '3	294	Safaei <i>et al.</i> (2011)
ureC (R)	5' GCTTACTTTCTAACACTAACGCGC '3		

## RESULTS

**Table 1:** Incidence of isolated *Helicobacter* species in the examined hen's eggs shell samples.

Type of samples	No. of samples analyzed	No. of Positive samples	%
Balady hen's egg shells	30	10	33.33
Poultry farms hen's egg shells	30	9	30

**Table 2:** Incidence of different isolated *Helicobacter* species recovered from the examined hen's eggs shell samples.

Isolated <i>Helicobacter</i> species	Percentage of positive shell samples			
	Balady		Farms	
	No./30	%	No./30	%
<i>H. pylori</i>	5	16.67	4	13.33
<i>H. cinaedi</i>	2	6.67	3	10
<i>H. felis</i>	2	6.67	2	6.67
<i>H. pullorum</i>	1	3.33	-	-
Total	10	33.33	9	30

**Table 3:** Incidence of isolated *Helicobacter* species in the examined hen's eggs content samples.

Type of samples	No. of samples analyzed	Positive samples	
		No.	%
Balady hen's egg contents	30	1	3.33
Poultry farms hen's egg contents	30	-	-

**Table 4:** Incidence of different isolated *Helicobacter* species recovered from the examined hen's eggs content samples.

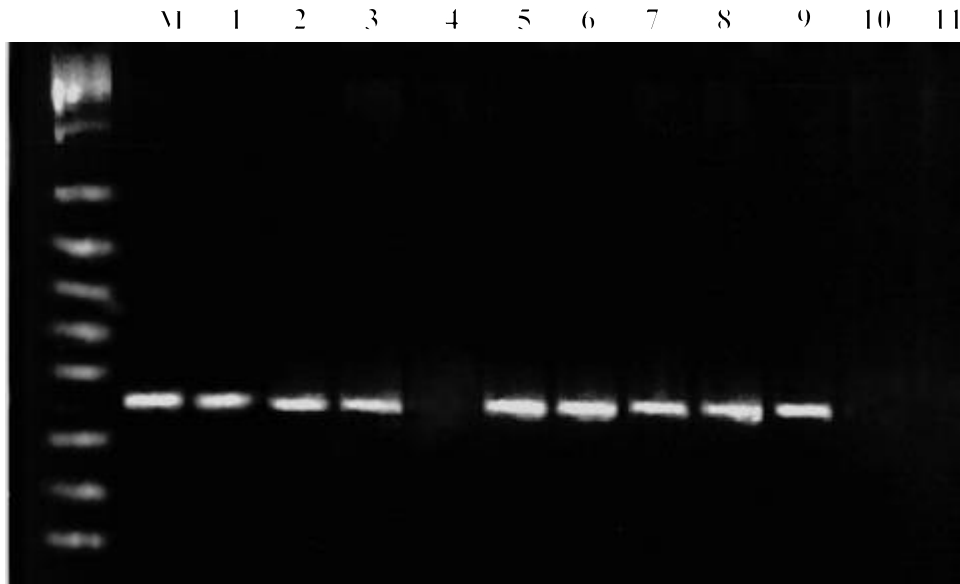
Isolated <i>Helicobacter</i> species	Positive egg content samples			
	Balady		Farms	
	No./30	%	No./30	%
<i>H. pullorum</i>	1	3.33	-	-
Total	1	3.33	-	-

**Table 5:** Comparison between the incidence of *H. pylori* in the examined hen's egg shell samples by using biochemical and PCR technique.

Examined samples	Positive samples			
	biochemical		PCR	
	No/30	%	No/30	%
Balady hen's egg shells	5	16.67	4	13.33
Poultry farms hen's egg shells	4	13.33	4	13.33
Total isolates	9	30	8	26.66

**Table 6:** Relation between biochemical and PCR technique in isolated *H. pylori*.

Examined samples	No. of examined <i>H. pylori</i>	Positive samples	
		No.	%
Balady hen's egg shells	5	4	80%
Poultry farms hen's egg shells	4	4	100%
<b>Total</b>	<b>9</b>	<b>8</b>	<b>88.89%</b>

**Photograph 1:** Agarose gel electrophoresis of PCR amplification products using *ureC* gene as specific primer for identification of *H. pylori*.

**Lane M:** 100 bp ladder as molecular DNA marker.

**Lane 1:** Control positive *H. pylori* for *ureC* gene.

**Lane 11:** Control negative for *H. pylori*.

**Lanes (2, 3, 4, 6, 7, 8, 9 and 10):** Positive strains as *H. pylori*.

**Lane 5:** Negative *H. pylori* isolated from balady egg shell samples.

**Lanes (2, 3, 6, and 9):** Strains isolated from balady egg shell samples.

**Lanes (4, 7, 8 and 10):** Isolated strains from poultry farms egg shell samples.

## DISCUSSION

The idea of the present study dealing with the incidence of *Helicobacter* species in hen's eggs was coming from the fact that the organism was originally isolated from the feces of broilers and laying hens (Burnens *et al.*, 1994, Stanley *et al.*, 1994). In addition, a preliminary study showed its presence on 60% of poultry carcasses pointing to it as a potentially important food-associated human pathogen.

Therefore, the discovery of *Helicobacter* species has sparked an interest in exploring the pathogenic potential of these organisms in food especially in eggs because eggs are considered as an essential food element for growth and maintenance of human health in addition to its high nutrient contents, low caloric value and ease of digestibility. However the nutrients that make eggs a high – quality food for human are

also a good medium for bacteria (Frazier and Westhoff, 1986).

It was observed from Table 1 that shells contamination with *Helicobacter* species on (HPSPA) in balady hens eggs was (33.33%) and in poultry farms eggs was (30%). This confirms the opinion of Burnens *et al.* (1994) and Stanley *et al.* (1994) that the contamination may come from feces of hens. The relatively high incidence of *Helicobacter* species contamination was observed in examined balady hen's eggs shell samples in percentage of (33.33%), and in percentage of (30%) in the examined egg shell samples from poultry farms hen's eggs. This could be attributed to the bad hygiene during the production of eggs. Also, eggs are liable to contamination from feces of hens according to (Burnens *et al.*, 1994, Stanley *et al.*, 1994) who stated that the organism was originally isolated from the feces of broilers and

laying hens. The *Helicobacter* species are recovered from the examined balady hen's egg content indicating the bad hygienic measures in the farmer's houses.

Data summarized in Table 1 indicated that the contamination of the balady hen's eggs shell samples was slightly higher than the contamination of the poultry farms egg shell samples. Presence of *Helicobacter* species in both types of egg indicate the bad hygienic measures of the production of hen's eggs. Data showed in Table 1 was less than the result obtained by Abdel Hameed and Sender (2011) indicating the good hygienic measures during production of eggs.

The external shell contamination could be important for the shell life and the food safety of consumption of eggs and egg's products (Smith *et al.*, 2000). This is observed from the obtained results in Table 2 that the identified *Helicobacter* species from examined balady hen's egg shells samples were *H. pylori* 5 (16.67%), *H. cinaedi* 2 (6.67%), *H. felis* 2 (6.67%) and *H. pullorum* 1 (3.33%). These results agree with those of Abdel Hameed and Amin (2010) who make the same protocol in isolation and identification of *Helicobacter* species as described by Stevenson *et al.* (2000). In the present results in Table 2 of investigated poultry farms hen's egg shells samples the isolated strains were identified as *H. pylori* 4 (13.33%), *H. cinaedi* 3 (10%) and *H. felis* 2 (6.67%).

Table 3 showed that 1 out of 30 examined fresh content of balady eggs samples were contaminated with *Helicobacter* species but the study failed to isolate any *Helicobacter* species from examined fresh contents of poultry farms hen's eggs. This may be due to competition effect or using antibiotics or due to other factors which need further investigation.

In the present study it was clear that incidence of different *Helicobacter* species in the examined hen's eggs content from both baladi and poultry farms hen's egg was less than the results obtained by Abdel Hameed and Amin (2010). This indicates the hygienic measures applied leading to minimizing egg content contamination.

Regarding to data presented in Table 4 *H. pullorum* was the identified *Helicobacter* species (3.33%) recovered from the examined balady hen's egg contents. On the other hand *Helicobacter* species failed to be detected in examined poultry farms hen's egg contents which agree with the results obtained by both Abdel Hameed and Amin (2010) and Abdel Hameed and Sender (2011). These results may be due to presence of natural inhibitory substances in the egg.

*H. pullorum* was isolated from the liver, duodenum, and caecum of broiler and layer chickens, and from humans with gastroenteritis. It is a non-gastric urease

negative *Helicobacter* species colonizing the lower bowel, and has been linked with enteritis and hepatitis in broiler chickens and laying hens and diarrhea, gastroenteritis, liver disease in humans. *H. pullorum* can be considered a food borne human pathogen (Fox *et al.*, 1998).

*H. cinaedi* has been found in association with enteritis, proctocolitis and asymptomatic rectal infections in humans (Franklin *et al.*, 1996).

Table 6 indicated that conventional method led to identification of 9 bacterial isolates as *H. pylori* 5 from balady hen's egg shells and 4 from poultry farms hen's egg shells. The percentage of *H. pylori* in examined balady egg shell samples by using PCR assay was 4 (80%) from 5 examined samples and was 4 (100%) from 4 examined poultry farms egg shell samples.

In the present study as shown in Table 6 the conventional method led to identification of 9 bacterial isolates as *H. pylori*, based on biochemical tests. 8 from 9 (88.88%) isolates were confirmed as *H. pylori* with PCR by detection of one PCR product on agarose gel that matched the predicted size and confirmed the actual size of 294 bp that corresponded to ureC gene region of the gene.

Electrophoresis analysis of PCR amplification products using genus specific primer (ureC gene) showed that (Photo1). (M) Ladder marker 100 bp; lanes (2,3,4,6,7,8,9,10) isolates positive for *H. pylori* where, lane (5) isolates negative for *H. pylori* which isolated from balady egg shell samples, lanes (2,3,6,9) isolated from balady egg shell samples and lanes (4,7,8,10) isolated from poultry farms egg shell samples. PCR amplification with the primer generated an expected size product with a length of 294 bp from 8 *H. pylori* strains tested (Photo1). The PCR assay employed in this work specifically targets a region of the ureC gene which has been shown to be unique and essential for growth of *H. pylori*. It has been reported that detecting this gene improves sensitivity and specificity of recognition of *H. pylori* in samples containing prokaryotic cells as well as many organic impurities (Safaei *et al.*, 2011).

Results of biochemical identification of *Helicobacter* species by using conventional method revealed that 9 isolate of samples (15%) out of 20 samples were identified as *H. pylori*, 5 (16.66%) strains recovered from balady egg shell samples and 4 (13.33%) strains from poultry farms hen's egg shell samples. By using PCR technique for identification of suspected isolates, the results revealed that 8 out of 20 samples isolated were identified as *H. pylori*, where 4 from balady egg shell samples and 4 from poultry farms hen's egg shells samples.

So as noticed from the present results, the specificity of PCR technique for identification of *H. pylori* that previously identified by using conventional technique was more accurate. Stanley *et al.* (1994) and Ceelen *et al.* (2006) obtained the same specificity of the protocol and its ability to discriminate between closely related species. The results of PCR of the isolated *H. pylori* were nearly similar to the results obtained by Abdel Hameed and Amin (2010) which confirms the specificity of PCR methods.

It is known that several factors have been implicated in egg contamination. Among these are feces of the birds, litter material, improper handling of the eggs by retailers, unhygienic conditions of the markets where these eggs are being sold, contaminated egg crates, packing and poor storage methods (Bruce and Drysdale, 1994). Others are cloths and hands of poultry workers, the environment, dust transporting marketing and weather conditions. Eggs in many stores were exposed to high temperature and low humidity which favors the growth of microbes, especially fungi and hence the rapid decrease in the quality of eggs.

## CONCLUSION

The results of this work emphasized that baladi hen's egg shells were contaminated by some *Helicobacter* species including *H. pylori*, *H. felis*, *H. pullorum* and *H. cinaedi*, and poultry farms hen's egg shells were contaminated by some *Helicobacter* species including *H. pylori*, *H. felis* and *H. cinaedi*, in different percentages. This reflects bad hygienic measurements of laying hens, which probably constitute the major source of eggs contamination. The incidence of *Helicobacter* species in balady egg shell samples was higher than that of poultry farms hens eggs shell samples. The incidence of *Helicobacter* species in egg shell samples was higher than that of egg content samples and that clarify the bad hygienic measurements of laying hens, which probably constitute the major source of eggs contamination. The results of this investigation showed that only one egg content sample from examined balady hen's egg was contaminated with *Helicobacter* species. The results of this work emphasized that the poultry farms egg content samples were free from any *Helicobacter* species.

Therefore, the aforementioned data proved that we must pay great attention to the problems of these pathogens in our foods. Consequently, more restriction and preventive measures should be taken to improve the quality of eggs and clean egg production to protect consumers from being infected by *Helicobacter* which require many precautions.

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## تواجد أنواع الهليكوباكتر مع أولوية خاصة للهليكوباكتر بيلوري في بيض الدجاج

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يعتبر البيض غذاء متكامل يحتوى على معظم العناصر الغذائية الأساسية التي يحتاجها جسم الإنسان في جميع المراحل العمرية وفي نفس الوقت يعتبر بيض الدجاج وسط جيد لنمو وتكاثر الميكروبات المختلفة التي تمثل خطورة على صحة الإنسان ومن هذه الميكروبات ميكروب الهليكوباكتر الذي تتمثل خطورته على صحة الإنسان فيما يسببه من التهاب وتقرح معدى ومعوى وفي النهاية قد يؤدي الى سرطانات. من هنا كان الاهتمام بتحديد مدى تواجد هذا الميكروب في بيض الدجاج من المطالب الضرورية لذا تضمنت الدراسة جمع ٣٠٠ بيضة عشوائية من بيض الدجاج بواقع ١٥٠ بيضة من بيض الدجاج البلدى و ١٥٠ بيضة من بيض دجاج المزارع لمعرفة مدى تواجد ميكروب الهليكوباكتر. أسفرت النتائج عن تواجد ميكروبات الهليكوباكتر في ١٠ عينات بنسبة (٣٣,٣٣٪) من قشرة بيض الدجاج البلدى و ٩ عينات بنسبة (٣٠٪) من قشرة بيض دجاج المزارع و بنسبة (٣,٣٣٪) من المحتوى الداخلى لبيض الدجاج البلدى وذلك باستخدام مستنبت *Helicobacter pylori special peptone agar*. تم عزل ميكروب الهليكوباكتر بيلورى من ٥ عينات بنسبة (١٦,٦٧٪) من عينات قشرة بيض الدجاج البلدى ومن ٤ عينات بنسبة (١٣,٣٣٪) من قشرة بيض دجاج المزارع. كما أمكن عزل ميكروب الهليكوباكتر سنيدى بنسبة (٦,٦٧٪) وبنسبة (١٠٪) من عينات قشرة بيض الدجاج البلدى وقشرة بيض دجاج المزارع على التوالي. وقد تم عزل ميكروب الهليكوباكتر فيليس بنسبة (٦,٦٧٪) في كل من عينات قشرة بيض الدجاج البلدى وقشرة بيض دجاج المزارع. وكذلك أمكن عزل ميكروب الهليكوباكتر بلورم من عينة واحدة بنسبة (٣,٣٣٪) في كل من عينات القشرة وعينات المحتوى الداخلى من بيض الدجاج البلدى. ومن ناحية أخرى فقد كانت عينات المحتوى الداخلى لبيض دجاج المزارع خالية تماماً من أى من هذه الميكروبات. التعرف على جين (ureC) للهليكوباكتر بيلورى عن طريق إستخدام تفاعل البلمرة المتسلسل. أسفرت النتائج عن تواجد ميكروبات الهليكوباكتر بيلورى بنسبة إيجابية (٨٨,٨٩٪). هذا وقد تم مناقشة النتائج مع ذكر الشروط الصحية الواجب إتباعها لمنع تلوث البيض بميكروب الهليكوباكتر وذلك لحماية صحة المستهلك. هذا وقد أوصت الدراسة ببذل المزيد من الجهد لزيادة إنتاج البيض بطريقة آمنة عن طريق إتباع الطرق الصحية فى إنتاج بيض الدجاج ومنع التلوث فى كل من بيض الدجاج البلدى والمزارع.