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RESEARCH ARTICLE

INCIDENCE OF *HELICOBACTER PYLORI* IN FOOD AND WATER IN DHAMAR GOVERNORATE / YEMEN

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ABSTRACT

This study was designed to determine the incidence of *H.pylori* in different types of food and water in Dhamar Governorate. Four hundred and twenty six samples (426) were collected included 114 Red meat, 100 Poultry meat, 100 Salad and 112 Water. The results revealed that the isolation rate of *H.pylori* from total samples was (11.7%), the highest rate of *H.pylori* isolation were found in red meat samples (15.8%), then poultry meat (13.0 %), water (11.6%), and salad (6.0 %). When we study the relationship between prevalence of *H.pylori* in food and water with months during the period of study. April, June and May take the more isolated rate (24.5%), (20.4%) and (16.4%) respectively, while the rate of isolation were seen decrease whenever moved away from these three months and in both directions. The results indicated that there is significant differences at ($p < 0.05$) between isolation rate of *H.pylori* from above samples and months. From this study we concluded that the incidence of *H.pylori* in food and water in Dhamar Governorate was high, also found that food and water have a significant role in transmission of *H.pylori* to human.

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INTRODUCTION

Helicobacter pylori is a Gram – negative, S- shaped or curved rod – like bacterium, it is a little more than 2 µm and sometimes as long as 3.5 µm, and from 0.5 to 1.0 µm in diameter. It is highly motile due to its multiple flagella that emerge from one of the rounded ends. This bacterium has copious amounts of urease and is microaerophilic (Archampong *et al*., 2015; Ranjbar *et al*., 2016). The main reservoir of Helicobacter pylori (Hp) is human, particularly the human stomach, and the common bacterial infection linked to disorders of the gastrointestinal tract, Hp infection is a public health issue. It is one of the world's most common human bacterial infections and associated with chronic gastritis, peptic ulceration and gastric cancer (Hedlund, 2012; Ma *et al*., 2016). Approximately 50% (over 3 billion) of the world populations are known to be infected with Helicobacter pylori, mainly in the developing countries making it one of the most controversial bacteria in the world (Kusters *et al*., 2006; Salih, 2009 ; Nair *et al*., 2016). Although H. pylori infection occurs worldwide, but significant differences in prevalence have been found both within and between countries.

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Generally, the overall prevalence is higher in countries of under developed regions, such as Africa and Asia, than in the more developed countries in Western Europe and North America. Overall, *H.pylori* prevalence is decreasing as a result of improved sanitary conditions and treatment procedures (Azevedo *et al*., 2007; Niknam *et al*., 2014). The prevalence of *H.pylori* infection varies from 7.3 % to 92.0 %, depending on age, geographic location, and socioeconomic status of the populations (Kamil and Abdul – Majeed, 2007; Korkoca *et al*., 2015). Several studies have shown that the prevalence of H. pylori is still high in most countries. In north European and North American populations, about one-third of adults are still infected, whereas in south and east Europe, South America, and Asia, the prevalence of H. pylori is often higher than 50%.((Ma and Wang, 2013). In Yemen the prevalence of *H.pylori* is very high according to previous studies. El – Gunaid *et al*., in 1991 found that there was a high prevalence of gastric *H.pylori* colonization (93%) in Yemeni patients. In addition, there was another study in Yemen (Gunaid *et al*., 1995) showed that the esophageal and gastric carcinoma accounted for as much as(6%) of all patients who had an upper gastrointestinal endoscopy over a period of one year. In 2003, Al –Shami has shown that the prevalence of *H.pylori* infection among patients underwent upper gastrointestinal endoscopy in Sana'a major hospital was very

high (99.6 %). Gunaid *et al*., in 2003 reported that the prevalence of *H.pylori* infection in dyspeptic patients in Yemen was (82.2 %). Moreover, seroprevalence of *H.pylori* antibodies was (9%) among Yemeni children (Al- Shamahy, 2005). Al - Makdad *et al*., 2013 mentioned that the prevalence of H. pylori infection in hospital patients, who undergone upper gastrointestinal endoscopy is very high (98.7%). Also Mayass, 2013 found that the total prevalence of *H.pylori* antibody in blood were (82.5 %), while the prevalence of *H.pylori* in stool according to *H.pylori* antigen test and *H.pylori* isolation were (18.5 %). Several modes of transmission of H. pylori have been described in the literature; these included direct contact between subjects, which is considered the most common mode, contaminated water sources and food, and less commonly iatrogenic transmission (during endoscopies and dental care) and zoonotic transmission (Salih, 2009; Talaei *et al*., 2015). Environmental or animal reservoirs were investigated as sources of *H.pylori* infection. Food, animals, and water sources have been suggested as reservoirs outside the human gastrointestinal tract, and *H.pylori* or its DNA was detected in each of these sources (Khalifa *et al*., 2010; Momtaz *et al*., 2014). Food is a plausible source of *H.pylori* infections (Vale and Vitor, 2010; Hedlund, 2012). Several studies address the role of food in the transmission of *H.pylori*. Food products analysed are mainly milk, meat and vegetables. Among these, milk products are the most studied, probably because the infection is mainly acquired during childhood and milk is mostly consumed during this period (Abdel-Latif *et al*., 2016).

The role of foods in the transmission of H. pylori is still unknown but there were several investigations which focused on the identification of this bacterium in various types of food samples. However, there is an increasing need for comprehensive studies emphasizing the significance of *H.pylori* as a food-borne pathogen (Duynhoven and Jonge, 2001; El Dairouty *et al*., 2016). Also numerous epidemiological studies have reported positive associations between untreated or faecally contaminated drinking water and incidence of *H.pylori* infection (Bellack *et al*., 2006; Doyle, 2012). Various findings support the hypothesis of *H.pylori* water-borne infection. Chen *et al*., 2005 and Yahaghi *et al*., 2014,, mentioned that individuals who consume raw vegetables are more likely to acquire *H.pylori*. The association of the infection with consumption of raw vegetables is an additional indirect evidence of the presence of *H.pylori* in water used for irrigation of these vegetables (Abdel-Latif *et al*., 2016). *H.pylori* infection in Yemen is primarily acquired in early childhood. Its transmission routes are debated, therefore the aims of this research were to Study the incidence of *H.pylori* in food and water in Dhamar Governorate and to determine the relationship between incidence of *H.pylori* in food and water with months during the period of study. Also the studying of the epidemiological data on *H.pylori* help in the establishing public health action that could halt transmission and therefore acquisition of the infection and aid the therapeutic program to eradicate the bacterium.

MATERIALS AND METHODS

Four hundred and twenty six (426) different food and water samples (114 Red meat, 100 poultry meat, 100 Salad, and 112 Water). Solid samples (Red meat, Poultry meat, and Salad) were collected from the central Dhamar slaughterhouse, retail markets, Restaurants and street venders in different places of Dhamar Governorate, the samples put in sterile cooled polyethylene bags, and kept in ice box with temperature approximately 4°C. Water samples were collected in sterile bottle from surface ground water sources and tap water, then all of the samples were transported to the laboratory (Whale *et al*., 2003; Aodha, 2009). In the laboratory the isolation of H. pylori from solid samples were done under sterile conditions by cut the samples into small pieces by sterile blades for liberation of adherent bacteria to the enrichment broth (Nader, 1998). From red meat, poultry meat, and salad 25 gm (as the optimal sample size) was soaked in 250 ml of normal saline. A 0.5 ml portion of the suspension was then placed in a 4.5-ml brain heart infusion broth with 7% horse serum at tube without antibiotics and enriched in a microaerophilic atmosphere (GasPack; Oxoid, Basingstoke, England) at 37°C for 3 to 7 days (Sa´enz, *et al*., 2000; Jiang and Doyle, 2002; Mayass, 2013). After that a selective modified Campy-blood agar plates and EYE agar plate was inoculated with 100 µl of the enriched suspension and incubated at 37°C in micro-aerobic condition by use candle jar and use Campy Gen (2.5L) in incubator for 4-10 days for colonies to appear.

Water sample was filtered through a 142 mm diameter 0.45µm pore sized nylon membrane. The membrane was transferred into a sterile 140mm petridish, 25ml phosphate buffered saline (PBS) was added and the organisms re suspended using a cell scraper to rub the surface of the membrane and elute the deposit from the surface, transferal of 10 ml into sterile universal and storage at 4°C for analysis, then centrifugation of 10ml sample at 4°C for 30mins, remove and discard 5ml supernatant. Then 20µl of sample were cultured onto modified campy- blood agar with Dent's supplement (DENT) under microaerobic conditions (O_2 5%, CO_2 5%, H2 2%, N2 88%) for 4-10 day (Mayass, 2013). The identification of *H.pylori* isolates were confirmed by Biochemical tests including (Catalase Test, Oxidase Test, Urease Test, Indole Test, Growth in 1% Glycine, Growth in 3.5% Sodium chloride, TSI Reaction, H2S Test, Cephalothin/ Nalidixic Acid Sensitivity/Resistance, Hippurate Hydrolysis Test (Table A).

Table (A):- Biochemical tests for the confirming of *H.pylori*

Biochemical Tests	Results
Catalase	Positive
Oxidase	Positive
Urease	Positive
Indole	Negative
Growth in 1% Glycine	Negative
Growth in 3.5% NaCl	Negative
H_2S Production in (TSI)	Negative
TSI with lead acetate paper	Positive
Nalidixic acid	Resistance
Cephalothin	Sensitive
Hippurate Hydrolysis	Negative

RESULTS

From 426 samples of different types of food and water, 50 (11.7 %) were gave a positive result for isolation of *H.pylori*. This result include 18 (15.8 %) positive samples from red meat, 13 (13.0%) positive samples from poultry meat, 6 (6.0%) positive samples from salad and 13 (11.6 %) positive samples from water (Table 1 and Figure 1). With ($p > 0.0\ 5$) there is no significant difference between isolation of *H.pylori* from different types of samples.

Table 1. Incidence of *H.pylori* in Food and water Samples

Type of Sample	Number of sample	Positive		Negative		P Value
		No.	%	No.	%	
Red meat	114	18	15.8	96	84.2	
Poultry meat	100	13	13.0	87	87.0	
Salad	100	6	6.0	94	94.0	0.16
Water	112	13	11.6	99	88.4	
Total	426	50	11.7	376	88.3	

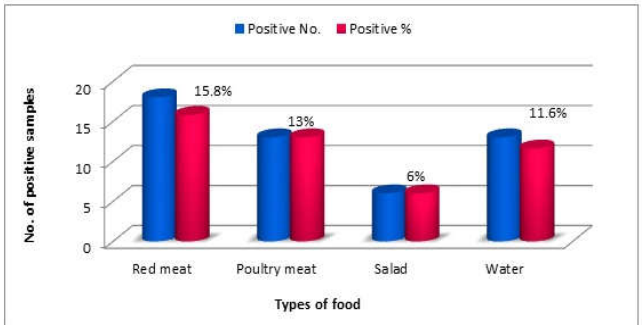

Figure 1. Incidence of *H.pylori* in Food and Water Samples

When study the relationship between Months and incidence of *H.pylori* in total samples of food and water during period of the research, we noticed that the highest rate of isolation were found in April 12 (24.5 %), approximately to this rate in June 10 (20.4 %), and in May 9 (16.4 %), but the rate of isolation were seen decrease whenever moved away from these three months and in both directions (Table 2 and Figure 2).

Table 2. Relationship between Months and incidence of *H.pylori* in Total Samples of Food and water during period of the Research

Months	No. of sample	Positive Sample		Negative Sample		P Value
		No.	%	No.	%	
February	49	3	6.1	46	93.9	
March	45	5	11.1	40	88.9	
April	49	12	24.5	37	75.5	
May	55	9	16.4	46	83.6	
June	49	10	20.4	39	79.6	
July	49	7	14.3	42	85.7	0.002
August	44	1	2.3	43	97.7	
September	42	0	0.0	42	100	
October	44	3	6.8	41	93.2	
Total	426	50	11.7	376	88.3	

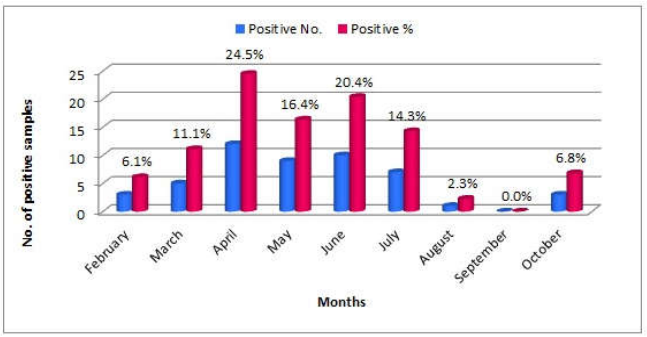

Figure 2. Relationship between Months and incidence of *H.pylori* in Total Samples of Food and water during period of the Research

Also the relationship between months and incidence of *H. pylori* in red meat, poultry meat, salad, and water were followed up. As shown in (Table 3 and Figure 3) the highest rate of isolation *H.pylori* from red meat were found in June 4 (33.3 %), approximately to this rate in April 4 (28.6%), but the rate of isolation were seen decrease whenever moved away from two months and in both directions.

Table 3. Relationship between Months and incidence of *H.pylori* in Red meat during period of the Research

Months	Number of samples	Positive		Negative		P Value
		No.	%	No.	%	
February	12	0	0.0	12	100	
March	13	3	23.1	10	76.9	
April	14	4	28.6	10	71.4	
May	14	3	21.4	11	78.6	
June	12	4	33.3	8	66.7	
July	15	3	20.0	12	80.0	0.14
August	13	1	7.7	12	92.3	
September	11	0	0.0	11	100	
October	10	0	0.0	10	100	
Total	114	18	15.8	96	84.2	

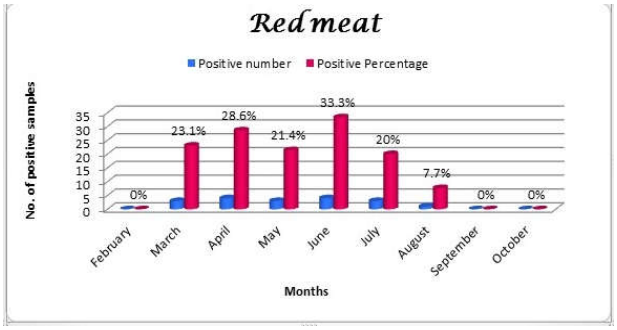

Figure 3. Relationship between Months and incidence of *H.pylori* in Red meat during period of the Research

Table 4 and Figure 4 illustrate that the highest rate of *H.pylori* isolation from poultry meat were found in May 5 (35.7%), but the rate of isolation were seen decrease whenever moved away from this month and in both directions until no isolation found in February, March, July, August, and September.

Table 4. Relationship between Months and incidence of *H.pylori* in Poultry meat during period of the Research

Months	Number of samples	Positive		Negative		P Value
		No.	%	No.	%	
February	12	0	0.0	12	100	
March	10	0	0.0	10	100	
April	12	3	25.0	9	75.0	
May	14	5	35.7	9	64.3	
June	13	3	23.1	10	76.9	0.03
July	10	0	0.0	10	100	
August	10	0	0.0	10	100	
September	9	0	0.0	9	100	
October	10	2	20.0	8	80.0	
Total	100	13	13.0	87	87.0	

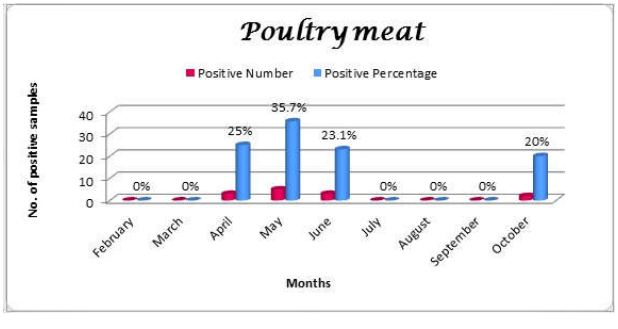

Figure 4. Relationship between Months and incidence of *H.pylori* in Poultry meat during period of the Research

The relationship between months and the incidence of *H.pylori* in Salad showed that the highest rate of isolation were found in June and July, 2 (18.2 %) and 2 (16.7 %) respectively, but the rate of isolation were seen decrease in October 1 (9.1 %), and in February 1 (8.3%), and no isolation found in March, April, May, August and September (Table 5 and Figure 5).

Table 5. Relationship between Months and incidence of *H.pylori* in Salad during period of the Research

Months	Number of samples	Positive		Negative		P Value
		No.	%	No.	%	0.34
February	12	1	8.3	11	91.7	
March	10	0	0.0	10	100	
April	10	0	0.0	10	100	
May	13	0	0.0	13	100	
June	11	2	18.2	9	81.8	
July	12	2	16.7	10	83.3	
August	10	0	0.0	10	100	
September	11	0	0.0	11	100	
October	11	1	9.1	10	90.9	
Total	100	6	6.0	94	94.0	

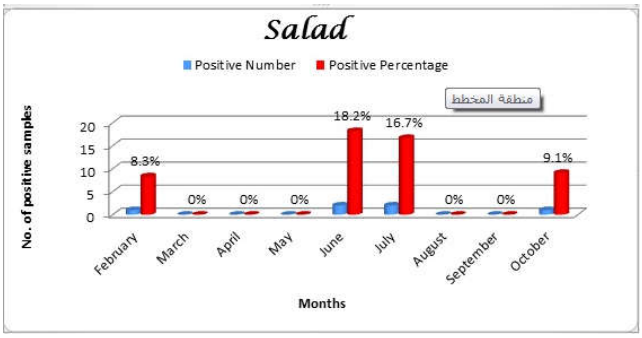

Figure 5. Relationship between Months and incidence of *H.pylori* in Salad during period of the Research

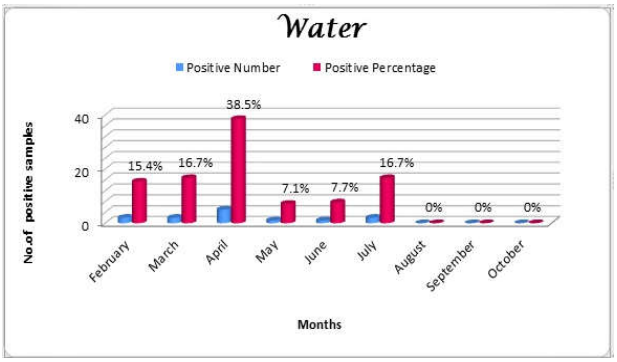

Figure 6. Relationship between Months and incidence of *H.pylori* in Water during period of the Research

Table 6. Relationship between Months and incidence of *H.pylori* in Water during period of the Research

Months	Number of samples	Positive		Negative		P Value
		No.	%	No.	%	
February	13	2	15.4	11	84.6	
March	12	2	16.7	10	83.3	
April	13	5	38.5	8	61.5	
May	14	1	7.1	13	92.9	
June	13	1	7.7	12	92.3	
July	12	2	16.7	10	83.3	
August	11	0	0.0	11	100	0.06
September	11	0	0.0	11	100	
October	13	0	0.0	13	100	
Total	112	13	11.6	99	88.4	

Table 6 and Figure 6, show that the highest rate of isolation from water was found in April 5 (38.5 %), but the rate of isolation were seen decrease whenever moved away from this month and in both directions and no isolation found in August, September and October.

DISCUSSION

Helicobacter pylori infection in humans is associated strongly with upper gastrointestinal diseases such as peptic ulcer diseases (gastric ulcer, duodenal ulcer) and malignancies (gastric cancer, lymphoma). *It* is believed to be transmitted primarily by faecal – oral or oral – oral routes, with food and water as possible vehicles of infection. Also *H.pylori* has been identified in different types of food and in water sources (Doyle, 2012; Saeidi and Sheikhshahrokh, 2016). In the study at hand, Four hundred and twenty six (426) different food and water samples were collected. These samples included 114 Red meat, 100 poultry meat, 100 Salad, and 112 Water, and the isolation rate of *Hp* from all samples were 50 (11.7 %). The highest rate of *Hp* was found in red meat samples 18 (15.8%), then poultry meat 13 (13.0 %), and water 13 (11.6%), while the lowest rate of isolation was from salad 6 (6.0 %) (Table 1 and Figure 1). Our result were consistent with Atapoor *et al*., 2014 in Iran, whom found that the total isolation rate of Hp from vegetables and salad samples were 44 out of 460 (9.56%) using the culture method, while the Polymerase Chain Reaction technique showed that 50 of 460 samples (10.86 %) were positive for Hp. Yahaghi *et al*., 2014 in Iran, found that 7 out of 50 (14.00 %) salad and 52 out of 380 (13.68%) vegetable samples harbored Hp. Also our result were compatible with the study conducted by (Talaei *et al.*, 2015), whom found that the frequency of *Hp* in ruminant raw milk samples were 28 out of 210 (13.33 %), and with (Kianpour *et al.*, 2014) in Iran, whom reported that the *H. pylori ureC* gene was detected in 24 (11.4%) of buffalo milk samples. On the other hand, Hemmatinezhad *et al.,* 2016 in Iran, revealed that from 550 ready to eat food samples, 74 (13.45 %) were positive for *Hp*. The most commonly contaminated samples were olvie salad (36 %), restaurant salad (30 %), fruit salad (28 %) and soup (22 %). There were no positive results for sausage, salami and chicken nugget.

Many searches were inconsistently with our finding Dore *et al*., 2001 in USA found that Hp were detected in 38/63 (60%) of milk samples and in 6/20 (30%) of sheep tissue samples. in the Chicago area Meng *et al.,* 2008 found that *Hp* were detected with multiplex polymerase chain reaction (PCR) in 4/11 (36 %) of the raw chickens and in 8/18 (44 %) of the ready-to-eat raw tuna meat. Rahimi and Kheirabadi, 2012 in Iran, reported that three of 447 milk samples (0.67%), including two sheep (2.2%) and one buffalo (1.6%) milk samples were found to be contaminated with *Hp* by using the cultural method, while *H.pylori ureC* gene was detected in 56 (12.5%) of milk samples, including 19 cow (14.1%), 11 sheep (12.2%), nine goat (8.7%), two camel (3.6%), and 15 buffalo (23.4%) milk samples. In the study conducted by Mousavi *et al*., 2014, they observed that 103 / 520 of milk samples (19.8%) and 77 / 400 dairy products samples (19.2%) were contaminated with Hp. The most frequently contaminated samples were ovine milk (35%) and traditional cheese (30%), and the prevalence of vacA, cagA, iceA and oipA factors were 75.0 %, 76.6%, 41.6% and 25.0%, respectively. El-Dairouty *et al.*, 2016 reported that the distribution of Hp in raw meat, raw

poultry meat, and luncheon meat were (3.3 %), (5.0 %), and (5.0 %) respectively. In another hand Saeidi and Sheikhshahrokh, 2016 illustrated that the distribution of Hp in total samples of raw milk and meat was 197 / 820 (24.02%), including 92/420 (21.90%) total raw milk and 105/ 400 (26.25%) total raw meat. When study the incidence of Hp in water, our results (11.6 %) consider lesser than results showed by Hegarty *et al.,* 1999 whom found that the actively respiring *Hp* were present in 25 of 42 (60%) of the surface water samples and 13 of 20 (65 %) of the shallow ground waters sampled in Pennsylvania and Ohio. Lu *et al.,* 2002 found that the isolation of *Hp* from water was 23 / 37 (62.2 %) isolates were confirmed to be *Hp* by 16S rRNA PCR. In North American rivers, the *Helicobacter species* were detected in 18 / 33 (55%) of river samples, from these river samples *Hp* was detected in 11/33 (33%) (Voytek *et al.,* 2005). The result of our study consider more than results showed in other research in Basra governorate, Iraq (Al - Sulami *et al.,* 2010) whom found that the total isolation rate of *Hp* from drinking water was 10 (2.0%). Khan *et al.,* 2012 in Karachi, Pakistan, noticed that 2/50 (4%) samples of drinking water collected from two different densely populated town areas were found to be positive for *Hp*. Ranjbar *et al.*, 2016 in iran found that 12/ 400 (3 %) of drinking water samples were positive for Hp. In several previous studies, there were differences in the prevalence rate of *H. pylori* infection between countries. It can be due to diverse contributing factors including socioeconomic status, geographical, living conditions or due to another conditions related with personal hygiene. According to our results and previous studies, *Hp* actually exist in foods. Thus, it is highly probable that *Hp* could contaminate foods and survive in or on these foods for some time, being transmitted to those who consume it. This supports the propounded oral-oral and fecal-oral modes of transmission. Methods for direct culture of *Hp* haven't been fully developed. part of the difficulty in detecting this pathogen is that changes in cell morphology, metabolism an?? growth patterns occur when the organism is exposed to different environmental stimuli, including the condition of viable but non-culturable organisms. Likewise, the isolation of *Hp* from food products is extremely difficult due to the presence of accompanying microflora and to the presumably very low *Hp* load (Vale and Vítor, 2010).

In this study, the relationship between months and incidence of Hp during period of research in Dhamar Governorate was studied. Table 2 and Figure 2 show that the highest rate of isolation of *Hp* from different kinds of food and water were found in April 12 (24.5 %), approximately close to this rate in June 10 (20.4 %), and in May 9 (16.4 %). But the rate of isolation were seen decrease whenever moved away from these three months and in both directions. It is noted that the Chi-square value was 24.62 in significant value (0.002) ($p<0.05$). There is significant difference between isolation rate of *H.pylori* from food and water with months during the period of the study, where it is noted that the more prevalence were found in April, June and May. As shown in (Table 3 and Figure 3), the highest rate of *Hp* isolation from red meat was found in June 4 (33.3%), approximately to this rate in April 4(28.6 %), but the rate of isolation was seen decrease whenever moved away from these two months and in both directions. With ($p > 0.05$) there is no significant difference between isolation of *Hp* from red meat and months. Table 4 and Figure 4, showed that the highest rate of *Hp* isolation from poultry meat was found in May 5 (35.7 %), but the rate of isolation was decrease whenever moved away from this month and in both directions until no isolation found in February, March, July, August, and September. There is significant differences between the isolation of *Hp* from poultry meat and months at ($p < 0.05$). From Table 5 and Figure 5, the incidence of *Hp* in salad increased in June, and July, the isolation rate were 2 (18.18 %), and 2 (16.67 %) respectively, but the rate of isolation were seen decrease in October 1 (9.09 %), and in February 1 (8.33 %), and no isolation found in March, April, May, August and September. There is no significant differences between the isolation of *Hp* from salad and months at ($p > 0.05$). According to the Table 6 and Figure 6, we noticed that the highest rate of *Hp* isolation from water was found in April 5 (38.5%), but the rate of isolation was seen decrease whenever moved away from this month and in both directions and no isolation found in August, September and October. Also there is no Significant difference between the isolation of *Hp* from water and months during the period of study at ($p > 0.05$). In this part of our study, we did not found any researches available about relationship between months and incidence of *Hp* in food and water during period of study to compare them with our results, but there were some studies about relationship between months and incidence of *Campylobacter* in food (Poultry meat) in Dhamar Governorate showed that the prevalence increased in June and July, isolation rate were (18.03 %) and (20.83 %) respectively (Al-Dulaimi, 2013). From this research we concluded that the incidence of Hp in food and water in Dhamar Governorate seems to be high (11.7 %), this high percentage may be due to socioeconomic status, family dietary, poor living conditions, and sanitary habits or another risk factors that can increase the occurrence of infection.

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