Determination of the Prevalence of Helicobacter bilis by PCR in Iranian Mus musculus

Fatemi Masih[1], Firoozinia Mohammad[2], Maryam Hazeri[2], Abbas Doosti[1]*

[1] Biotechnology Research Center, Islamic Azad University, Shahrekord Branch, Iran, Email: masieh_fatemi_esf@yahoo.com
[2]Institute of Bioscience, University Putra Malaysia, Kuala Lumpur 43300, Malaysia, Email: mohammadf8623@yahoo.com, maryamhazeri.1983@gmail.com
*Corresponding author: Abbas Doosti, Biotechnology Research Center, Islamic Azad University, Shahrekord, Iran. Email: doosti.abbas@yahoo.com

Abstract: Helicobacter bilis is a member of the murine enterohepatic Helicobacter spp which identified and isolated from bile, liver, and intestine of aged, inbred mice and associated with liver and Hepatobiliary tract disease in human patients. The aim of the present study was to investigation of the presence of Helicobacter species, particularly Helicobacter bilis for the first time in liver of Mus musculus in Isfahan province (center of Iran) by the molecular method. DNA was extracted from 300 wild mice liver specimen using the DNA Extraction Kit and Nested PCR was performed on template DNA and amplified products were separated on 1.5% agarose gel. From 300 liver samples which assayed, 204 (68%) samples were positive for genus Helicobacter and 118 (39.33%) samples were positive for Helicobacter bilis ($P \leq 0.05$). In conclusion, PCR technique can detect H. bilis DNA in liver samples of Mus musculus in the center of Iran and wild mice may be carrying zoonosis infection such as H. bilis. According to the results of this study, wild mice may be important source and the potential of carrying Zoonosis infection such as H.bilis. These data suggest Mus musculus may have the potential of carrying H.bilis and zoonotic microorganisms that can be infectious in humans.

[Fatemi Masih, Firoozinia Mohammad, Maryam Hazeri, Abbas Doosti. **Determination of the Prevalence of Helicobacter bilis by PCR in Iranian Mus musculus**. *Life Sci J* 2013;10(12s):696-702] (ISSN:1097-8135). http://www.lifesciencesite.com. 111

Keywords: Helicobacter bilis**,** Nested PCR**,** Mus musculus, Iran

Introduction

Zoonosis diseases which harmful to human and animal health are caused by infectious agents that may be transmitted from vertebrate animals to humans and vice versa (Taylor et al. 2001; Hubalek 2003).

Mus musculus and laboratory mice that have been exposed to wild mice may have the potential of carrying a wide-ranging of zoonotic microorganisms that can be infectious in human (Mushtaq-ul-Hassan et al, 2008). In Helicobacteriosis appears to be a common identified in rodents, the infections and infestations present in rodent could serve as a source of infection or infestation zoonotic disease potential. For the first time, Helicobacter pylori (H. pylori) from human were discovered by Warren and Marshall in 1983 (Warren and Marshal 1983). Up to now, 33 spiral-shaped bacteria of Helicobacter spp. have been isolated from stomach, intestinal tracts, livers and bile duct of mammals (e.g. human) and birds. (http://www.bacterio.cict.fr).

Some Helicobacter spp., are resistant to bile and recently the correlation of Helicobacter pylori and some of enterohepatic Helicobacter spp. such as Helicobacter bilis (H.bilis), to Hepatobiliary diseases was demonstrated in human and animals but roles of them in pathogenesis are under investigation (Avenaud et al. 2000; Chen et al. 2003; Apostolov et al. 2005). For the first time, Fox et al. in 1995; identified and isolated H. bilis from bile, liver, and intestine of aged, inbred mice. Recently, many studies have focused on the roles of H. bilis pathogenesis in human and animal and isolation of it from human, rats, dogs, and gerbils (Eaton et al. 1996; Shomer et al. 1997; Shomer et al. 1998; Franklin et al. 1998; Fox et al. 1998a; Fox et al. 1998b; Goto et al. 2000; Ge et al. 2001; Matsukura et al. 2002; Fox et al. 2004). H. bilis is a member of the murine enterohepatic Helicobacter spp., Gram negative, and fusi-form bacterium (Fox et al. 1995). It has 3 to 14 multiple bipolar flagella and is motile micro-organism which is highly fastidious organism and grew in 42 as well as 37°C under microaerophilic atmosphere conditions same as other Helicobacter species, and exhibited a nitrate reduction to nitrite, uresae, catalase (Fox et al. 1995). H. bilis is resistant to both Cephalothin and Nalidixic acid (30 µg disk) but it is sensitive to Metronidazole (Fox et al. 1995). Several studies were confirmed the association of H. bilis with liver and hepatobiliary tract diseases in human patients (Matsukura et al. 2002; Murata et al. 2004; Kobayashi et al. 2005; Takayama et al. 2010).

Various methods were used for diagnosis of H. bilis such as fecal culture, polymerase chain reaction (PCR) nucleic acid amplification, membrane extract

enzyme-linked immunosorbent assay (ELISA), and histological examination (Hodzic et al., 2001; Feng et al., 2004; Feng et al,. 2005). The aim of the present study was the determination of the frequency of H. bilis in liver of mus musculus collected from Isfahan province (center of Iran) using the Nested PCR assay.

Materials and Methods

All procedures performed on live animals described in this report were approved by the ethics committee of each institution.

Trapping and Animal Collection

300 wild mice were collected between summer and winter 2011 by trapping. Sherman-style aluminum folding live traps and glue traps were placed in separate animal facilities including hallways, garages, loading docks, and feed and bedding rooms in from six interurban areas of Isfahan province (Isfahan, Najafabad, Fereydunshahr, Mobarakeh, Golpayegan, and Kashan),. Traps were baited with peanut butter, an apple slice and beef bits provide a source of food and water. Traps were set Saturday evening and taken up on Wednesday by noon and cage placement was documented on a checklist and checked twice daily when in place.

Necropsy and Sample Collection

Live mice were transported in cardboard containers to the Biotechnology Research Center of Islamic Azad University of Shahrekord Branch. Resources necropsy area, upon arrival at our institution, the mice were authorized by inhalation of CO2 within 24 h of entrapment in CO2 chamber. All animals were handled according to approved protocols.

Sample Collection

Liver specimens were removed and collected aseptically at necropsy immediately after euthanasia and frozen at –20 °C until DNA extraction.

DNA Extraction From Liver Tissues

Frozen hepatic tissues were thawed and homogenized, and DNA was extracted from 30–35 mg/specimen using the QIAamp DNA Kit (QIAGen, Hilden, Germany) according to the manufacturer's instructions with minor modifications. DNA was stored at –20 °C following concentration and purity assessment using a spectrophotometer (name of Instrument Company) at OD 260/ 280. Furthermore, DNA was extracted from the reference type strains H. bilis (ATCC 4387) and cecum of mouse autopsy that were positive for H. bilis and they used as positive controls for the development and validation of Helicobacter species-specific Polymerase Chain Reaction (PCR).

Amplification Conditions

Nested PCR was performed on the template DNA from liver samples using H. bilis specific primers. Amplification was carried out using the Mastercycler Gradient Thermal Cycler (Eppendorf, Germany). The first PCR reaction performed using Helicobacter universal primers for the DNA samples.

All oligonucleotide primers were synthesized by CinaGene (CinaGene Co, Tehran, Iran). The oligonucleotide Helicobacter universal primers forward 5'-CTATGACGGGTATOCGGC-3' and reverse 5'-CTCACGACACGAGCTGAC-3' described by Hamada et al. (Hamada et al, 2009) (product size 781bp). In the first step, PCR reaction was performed in a total volume of 25 µL in 0.5 ml tubes containing 1 µg of the extracted DNA sample, 1 µM of each primers, 2 mM Mgcl2, 200 µM dNTP, 2.5 µL of 10X PCR buffer (10 mM Tris-HCl, 1.5 mM MgCl2, 50 mM KCl [pH 8.3]) and 1 unit of Taq DNA polymerase (Roche applied science, Germany). This solution was initially denatured at 95°C for 5 minutes, followed by 35 cycles of denaturation (94°C for 30 second), annealing (55°C for 30 second), elongation (72°C for 1 minute) and a final extension at 72°C for 5 minutes. Oligonucleotide primers 5'-CAGAACTGCATTTGAAACTAC-3' and 5'-AAGCTCTGGCAAGCCAGC -3' described by Hamada et al. 22 (product size 418bp) were used as forward and reverse primers, respectively for detection of H. bilis in the second round of PCR reaction. 2 µL of PCR products from the first PCR were used as template DNA, and mix with condition described in the first step. This solution was initially denatured at 95°C for 5 minutes, followed by 30 cycles of denaturation (94°C for 30 seconds), annealing (55°C for 30 seconds), elongation (72°C for 1 minute) and a final extension at 72°C for 5 minutes. 10 µL of the amplified products were electrophoretically separated on 1.5% agarose gel (AgaroseTopVision LM GQ by CinaGene Co, Tehran, Iran). DNA ladders (GeneRuler™ 100 bp Plus DNA Ladder Fermentas, Germany) were used for each gel to determine amplified product size. Constant voltage of 80 V for 30 min was used for product separation. After electrophoresis, the gel was stained with ethidium bromide and images were obtained by UVIdoc gel documentation systems (UK). No band was constantly obtained when the extracted DNA sample was replaced by distilled water as a negative control

Statistical Analysis

Data were analyzed with a statistical software package, version 18 (SPSS Inc., Chicago, IL, USA).

The data were processed with the Chi - square test and P≤0. 05 was considered as a significant difference.

Result

Six interurban areas Mus musculus have been investigated in Isfahan province of Iran for the presence of H. bilis infection (Isfahan, Najafabad, Fereydunshahr, Mobarakeh, Golpayegan, and Kashan). The 16s rRNA gene of genus Helicobacter and H. bilis was successfully amplified with the forward and reverse primers using in nested PCR. Out of 300 liver samples which assayed by nested PCR in this research, 204 (68%) samples were positive in the first step of PCR (781bp fragment). The apparent prevalence frequency of Helicobacter was 72% (31 out of 50) in Isfahan, 66% (33 Out of 50) in Fereydunshahr, 48% (38 out of 50) in Mobarakeh , 72% (36 Out of 50) in Kashan, 58% (29 out of 50) in Najafabad and 74% (37 out of 50) in Golpayegan (Figure 2).

Only 118 (39.33%) samples were positive for H. bilis in second step of PCR (418bp fragment). So the rate of H. bilis infection in Isfahan, Najaf Abad, Fereydunshahr, Mobarakeh, Golpayegan and Kashan are 16 (32%), 21 (42%), 18 (36%), 24 (48%), 20 (40%) and 19 (38%), respectively. Agarose gel electrophoresis of the second step of PCR amplified products are shown in figure one.

These findings showed that, there were significant differences among the Mus musculus trapped in relation to interurban areas of Isfahan province distribution using chi-square test (P≤0. 05).

Until now, several species of the genus Helicobacter have been found in various mammals and birds, and some species have been confirmed to be causative agents in various infection and tissue inflammatory diseases of the stomach, intestine, gallbladder and the liver in human and mammals animals and birds (Stanley et al. 1994; Feng et al. 2002).

There is growing evidence bile-tolerant Helicobacter species, such as H. bilis (Fox et al. 1995; Murata et al. 2004; Takayama et al. 2010), H. cholecystus (Franklin et al. 1996; Matsukura et al. 2002; Nath et al. 2010), H. hepaticus (Fox et al. 1994; Ward et al. 1994; Nilsson et al. 2000; Pradhan and Dali 2004; Kobayashi et al. 2005; Gracia et al. 2001), Helicobacter pullorum (H. pullorum)(Stanley et al. 1994; Kornilovska et al. 2001; Pellicano et al. 2004; Celeen et al. 2005), and Helicobacter cholecystus (Franklin et al. 1996; Sturegård et al. 2004) residing in the biliary tree and liver tissue gastroduodenal tract in human as well as animal, and were thought to be associated with hepatic disease (Stanley et al. 1994; Rice 1995; Fox et al. 1995).

Discussion

H. bilis is thought to be one cause of the hepatitis and inflammatory bowel disease in mice (Shomer et al. 1997).

Fukuda et al and Matsukura et al found the H. bilis in gallbladder cancers and extra-hepatic biliary tract cancers in Japanese and Thai patients. They suggested that H. bilis plays a role in bile duct and gallbladder cancer (Matsukura et al. 2002; Fukuda et al. 2002).

H. bilis infection in biliary tract cancer (Murata et al. 2004) and H. bilis, Helicobacter rappini, and Helicobacter pullorum infection in frozen bile samples and gallbladder tissues from Chilean patients with chronic cholecystitis undergoing cholecystectomy (Fox et al. 1998) were found in two separate studies by PCR analysis.

Takayama and colleagues showed that H. bilis infection in a human bile duct cancer cell line activates transcript factors such as NF-jB that stimulate production of VEGF and lead to enhancement of angiogenesis and suggested H. bilis infection may be play an important role in malignancies in the biliary tract (Takayama et al. 2010).

Recently, bile-resistant Helicobacter species DNA was found in liver, cecum, and feces and intestinal of many kinds of vertebrate animals, using molecular biology techniques.

Because the nested PCR analysis was more sensitive than other method for detection of the Helicobacter spp., nested PCR technique was used in this study for the detection and identification of H. bilis as previously described (Hamada et al. 2009). Other Helicobacter spp. which isolated from the liver of animals include Helicobacter canis from a dog with acute hepatitis (Fox et al. 1996), H. pullorum from chicken with hepatitis (Stanley et al,. 1994), Helicobacter marmotae from woodchucks (Fox et al. 2002), Helicobacter mastomyrinus (H. mastomyrinus) from Rodents (Shen et al. 2005), Helicobacter cinaedi from rhesus macaques (Fox et al. 2001) and furthermore Helicobacter hepaticus has been observed in the livers of barrier maintained mice with multifocal necrotic hepatitis and pathogenetic role of it in liver disease has been documented in animals (Fox et al. 1996). It is to be noted that mentioned studies suggested that bile resistant Helicobacter organisms with Zoonosis disease's potential may also cause hepatitis in humans and other vertebrate animals.

In this study, we used specific primers for the 16S rRNA gene of genus Helicobacter and H. bilis to assess the presence of H. bilis DNA in liver samples from 300 wild mice, collected in Isfahan province. The 16S rRNA gene is highly conserved among

various species of Helicobacter, and the primer sets we used have been well characterized and used previously for detection of H. bilis (Hamada et al. 2009). Interestingly, in our study, Helicobacter DNA and H. bilis DNA was found in 68% and 39.33% the liver of Mus musculus, respectively. These results confirmed that Helicobacter spp. are able to infect the wild mice liver.

The pathogenic role of H. bilis is excluded in our study, however, the presence of these bacteria in wild mice suggests that they could be a source of H. bilis that are either potentially pathogenic in some laboratory mouse strains or that could interfere with experimental the possible role of these new Helicobacter species in Zoonosis diseases. However, to determine whether H. bilis is definitely a risk factor for the infected liver and tumor genesis, it is necessary to culture live H. bliss directly from the liver, but various bile-tolerant Helicobacter species are fastidious organisms and often difficult to culture, though cultural studies for these bacteria gave negative results.

In conclusion, PCR technique can detect H. bilis DNA in liver samples of Mus musculus in the center of Iran. To the best of our knowledge, this is the first report on the detection of H.bilis on Mus musculus liver in Isfahan province. We hypothesize that other possible Hepatobiliary Helicobacter species, such as H. hepaticus and the more recently discovered Helicobacter rodentium and H. mastomyrinus are one of the possibilities is that samples not identified to the species level by PCR (Rice 1995; Myles et al. 2004; Shen et al. 2005). According to the results of this study, wild mice may be important source and the potential of carrying Zoonosis infection such as H.bilis.

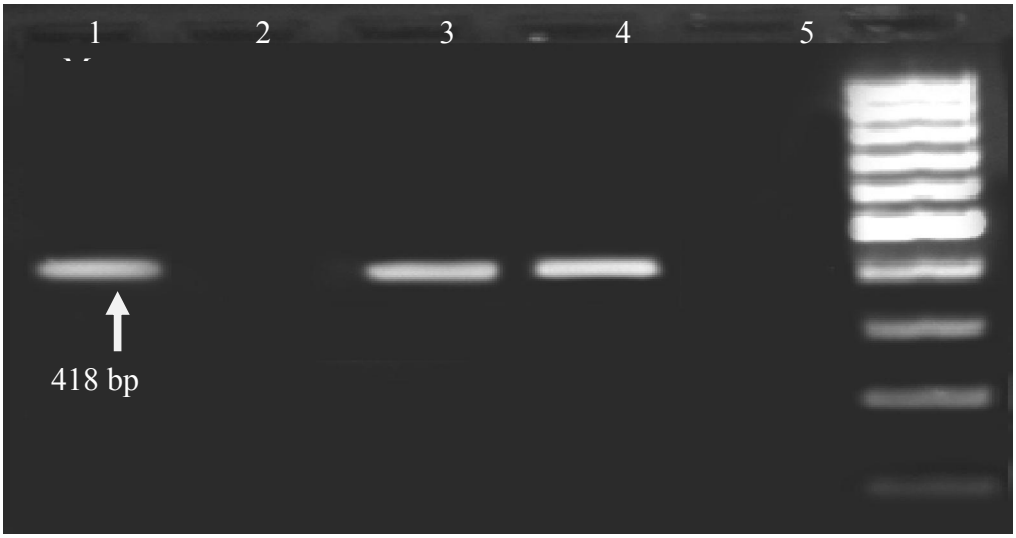

Figure 1: Identification of H. bilis by nested PCR amplification of the 16S rRNA gene. Lanes 1 and 2 are positive and negative controls, respectively. Lanes 3 and 4 are positive samples of H. bilis , lane 5 is negative sample. M, 100 bp DNA ladder (Fermentas, Germany)

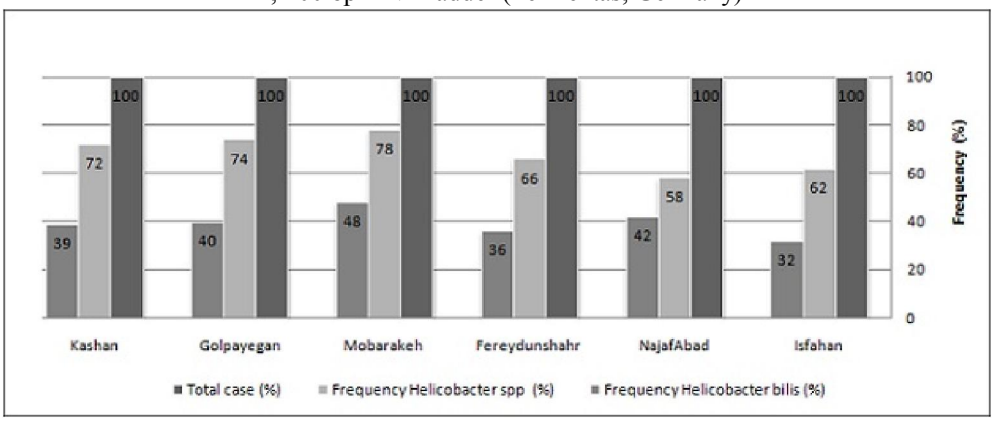

Figure 2: Frequency of Helicobacter spp. and Helicobacter bilis infection in mus musculus liver among six interurban areas of Isfahan province.

Acknowledgements

The authors wish to thank to the staff of the Biotechnology Research Center of Islamic Azad University of Shahrekord Branch in southwest Iran.

Corresponding Author:
Abbas Doosti
Biotechnology Research Center, Islamic Azad University, Shahrekord, Iran. Email: doosti.abbas@yahoo.com

References

1. Apostolov, E., Al-Soud, W.A., Nilsson, I., Kornilovska, I., Usenko, V., Lyzogubov, V., et al. Helicobacter pylori and other Helicobacter species in gallbladder and liver of patients with chronic cholecystitis detected by immunological and molecular methods. Scandinavian journal of gastroenterology 2005;**40(1)**, 96-102
2. Avenaud, P., Marais, A., Monteiro, L., Le Bail, B., Bioulac Sage, P., Balabaud, C., et al. Detection of Helicobacter species in the liver of patients with and without primary liver carcinoma. Cancer 2000;**89(7)**, 1431-9
3. Ceelen, L., Decostere, A., Verschraegen, G., Ducatelle, R. and Haesebrouck, F. Prevalence of Helicobacter pullorum among patients with gastrointestinal disease and clinically healthy persons. Journal of clinical microbiology 2005;**43(6)**, 2984
4. Chen, W., Li, D., Cannan, R. and Stubbs, R. Common presence of Helicobacter DNA in the gallbladder of patients with gallstone diseases and controls. Digestive and Liver Disease 2003;**35(4)**, 237-43
5. Eaton, K., Dewhirst, F., Paster, B., Tzellas, N., Coleman, B., Paola, J., et al. Prevalence and varieties of Helicobacter species in dogs from random sources and pet dogs: animal and public health implications. Journal of clinical microbiology 1996;**34(12)**,3165
6. Feng, S., Hodzic, E., Kendall, L.V., Smith, A., Freet, K. and Barthold, S.W. Cloning and expression of a Helicobacter bilis immunoreactive protein. Clinical and diagnostic laboratory immunology 2002;**9(3)**, 627-32
7. Fox, J., Dewhirst, F., Tully, J., Paster, B., Yan, L., Taylor, N., et al. Helicobacter hepaticus sp. nov., a microaerophilic bacterium isolated from livers and intestinal mucosal scrapings from mice. Journal of clinical microbiology 1994;**32(5)**, 1238
8. Fox, J., Drolet, R., Higgins, R., Messier, S., Yan, L., Coleman, B., et al. Helicobacter canis isolated from a dog liver with multifocal necrotizing hepatitis. Journal of clinical microbiology 1996;**34(10)**, 2479
9. Fox, J.G., Dewhirst, F.E., Shen, Z., Feng, Y., Taylor, N.S., Paster, B.J., et al. Hepatic Helicobacter species identified in bile and gallbladder tissue from Chileans with chronic cholecystitis. Gastroenterology 1998;**114(4)**, 755-63
10. Fox, J.G., Rogers, A.B., Whary, M.T., Taylor, N.S., Xu, S., Feng, Y., et al. Helicobacter bilis-associated hepatitis in outbred mice. Comparative Medicine 2004;**54(5)**, 571-7
11. Fox, J., Handt, L., Sheppard, B., Xu, S., Dewhirst, F., Motzel, S., et al. Isolation of Helicobacter cinaedi from the colon, liver, and mesenteric lymph node of a rhesus monkey with chronic colitis and hepatitis. Journal of clinical microbiology 2001;**39(4)**, 1580
12. Fox, J., Shen, Z., Feng, F., Dufover, J., Kaplan, M. and Dewhirst, F. Hepatobiliary Helicobacter spp. identified from patients with primary sclerosing cholangitis. Gastroenterology 1998;**114**, A978-A
13. Fox, J.G., Shen, Z., Xu, S., Feng, Y., Dangler, C.A., Dewhirst, F.E., et al. Helicobacter marmotae sp. nov. isolated from livers of woodchucks and intestines of cats. Journal of clinical microbiology 2002;**40(7)**, 2513
14. Fox, J.G., Yan, L.L., Dewhirst, F.E., Paster, B.J., Shames, B., Murphy, J.C., et al. Helicobacter bilis sp. nov., a novel Helicobacter species isolated from bile, livers, and intestines of aged, inbred mice. J Clin Microbiol 1995;**33(2)**, 445-54
15. Fox, J., Yan, L., Dewhirst, F., Paster, B., Shames, B., Murphy, J., et al. Helicobacter bilis sp. nov., a novel Helicobacter species isolated from bile, livers, and intestines of aged, inbred mice. Journal of clinical microbiology 1995;**33(2)**, 445
16. Franklin, C.L., Riley, L.K., Livingston, R.S., Beckwith, C.S., Besch-Williford, C.L., Hook, J., et al. Enterohepatic lesions in SCID mice infected with Helicobacter bilis. Comparative Medicine 1998;**48(4)**, 334-9
17. Franklin, C.L., Beckwith, C.S., Livingston, R.S., Riley, L.K., Gibson, S.V., Besch-Williford, C.L., et al. Isolation of a novel Helicobacter species, Helicobacter cholecystus sp. nov., from the gallbladders of Syrian hamsters with cholangiofibrosis and centrilobular pancreatitis. Journal of clinical microbiology 1996;**34(12)**, 2952
18. Fukuda, K., Kuroki, T., Tajima, Y., Tsuneoka, N., Kitajima, T., Matsuzaki, S., et al. Comparative analysis of Helicobacter DNAs and biliary

pathology in patients with and without hepatobiliary cancer. Carcinogenesis 2002;**23(11)**, 1927
19. García, A., Zeng, Y., Muthupalani, S., Ge, Z., Potter, A., Mobley, M.W., et al. Helicobacter hepaticus–Induced Liver Tumor Promotion Is Associated with Increased Serum Bile Acid and a Persistent Microbial-Induced Immune Response. Cancer Research 2011;**71(7)**, 2529
20. Ge, Z., Doig, P. and Fox, J.G. Characterization of proteins in the outer membrane preparation of a murine pathogen, Helicobacter bilis. Infection and immunity 2001;**69(5)**, 3502
21. Goto, K., Ohashi, H., Takakura, A. and Itoh, T. Current status of Helicobacter contamination of laboratory mice, rats, gerbils, and house musk shrews in Japan. Current microbiology 2000;**41(3)**,161-6
22. Hamada, T., Yokota, K., Ayada, K., Hirai, K., Kamada, T., Haruma, K., et al. Detection of Helicobacter hepaticus in human bile samples of patients with biliary disease. Helicobacter 2009;**14(6)**, 545-51
23. Hubálek, Z. Emerging human infectious diseases: anthroponoses, zoonoses, and sapronoses. Emerging infectious diseases 2003;**9(3)**, 403
24. Kobayashi, T., Harada, K., Miwa, K. and Nakanuma, Y. Helicobacter genus DNA fragments are commonly detectable in bile from patients with extrahepatic biliary diseases and associated with their pathogenesis. Digestive diseases and sciences 2005;**50(5)**, 862-7
25. Kornilovska, I., Nilsson, H. and Abu Al-Soud, W. Helicobacter pylori and Helicobacter pullorum in patients with cholelithiasis. Gut 2001;**49(Suppl II),** A68
26. Matsukura, N., Yokomuro, S., Yamada, S., Tajiri, T., Sundo, T., Hadama, T., et al. Association between Helicobacter bilis in bile and biliary tract malignancies: H. bilis in bile from Japanese and Thai patients with benign and malignant diseases in the biliary tract. Cancer Science 2002;**93(7)**, 842-7
27. Murata, H., Tsuji, S., Tsujii, M., Fu, H., Tanimura, H., Tsujimoto, M., et al. Helicobacter bilis infection in biliary tract cancer. Alimentary pharmacology & therapeutics 2004;**20**, 90-4
28. Mushtaq-ul-Hassan, M., Hussain, I., Shehzadi, B., Shaheen, M., Mahmood, M., Rafique, A., et al. Occurrence of some zoonotic microorganisms in faecal matter of house rat (Rattus rattus) and house mouse (Mus musculus) trapped from various structures. Pakistan Vet J 2008;**28(4)**, 171-4
29. Myles, M.H., Livingston, R.S. and Franklin, C.L. Pathogenicity of Helicobacter rodentium in A/JCr and SCID mice. Comparative Medicine 2004;**54(5)**, 549-57
30. Nath, G., Gulati, A.K. and Shukla, V.K. Role of bacteria in carcinogenesis, with special reference to carcinoma of the gallbladder. World Journal of Gastroenterology(WJG) 2010;**16(43)**, 5395
31. Nilsson, I., Lindgren, S., Eriksson, S. and Wadström, T. Serum antibodies to Helicobacter hepaticus and Helicobacter pylori in patients with chronic liver disease. Gut 2000;**46(3)**, 410
32. O'ROURKE, J., Grehan, M. and Lee, A. Non-pylori Helicobacter species in humans. Gut 2001;**49(5)**, 601
33. Pellicano, R., Mazzaferro, V., Grigioni, W.F., Cutufia, M.A., Fagoonee, S., Sileno, L., et al. Helicobacter species sequences in liver samples from patients with and without hepatocellular carcinoma. World Journal of Gastroenterology 2004;**10(4)**, 598-601
34. Pradhan, S. and Dali, S. Relation between gallbladder neoplasm and Helicobacter hepaticus infection. Kathmandu Univ Med J (KUMJ) 2004;**2(4)**, 331-5
35. Rice, J.M. Helicobacter hepaticus, a recently recognized bacterial pathogen, associated with chronic hepatitis and hepatocellular neoplasia in laboratory mice. Emerging infectious diseases 1995;**1(4)**, 129
36. Shen, Z., Xu, S., Dewhirst, F.E., Paster, B.J., Pena, J.A., Modlin, I.M., et al. A novel enterohepatic Helicobacter species 'Helicobacter mastomyrinus' isolated from the liver and intestine of rodents. Helicobacter 2005;**10(1)**, 59-70
37. Shomer, N.H., Dangler, C.A., Marini, R.P. and Fox, J.G. Helicobacter bilis/Helicobacter rodentium co-infection associated with diarrhea in a colony of scid mice. Comparative Medicine 1998;**48(5)**, 455-9
38. Shomer, N.H., Dangler, C.A., Schrenzel, M.D. and Fox, J.G. Helicobacter bilis-induced inflammatory bowel disease in scid mice with defined flora. Infection and immunity 1997;**65(11)**, 4858
39. Stanley, J., Linton, D., Burnens, A.P., Dewhirst, F.E., On, S.L.W., Porter, A., et al. Helicobacter pullorum sp. nov.-genotype and phenotype of a new species isolated from poultry and from human patients with gastroenteritis. Microbiology 1994;**140(12)**, 3441-9
40. Shames, B., Fox, J.G., Dewhirst, F., Yan, L., Shen, Z. and Taylor NS. Identification of widespread Helicobacter hepaticus infection in

feces in commercial mouse colonies by culture and PCR assay. Journal of clinical microbiology 1995;**33(11)**, 2968
41. Seymour, C., Lewis, R., Kim, M., Gagnon, D., Fox, J., Dewhirst, F., et al. Isolation of Helicobacter strains from wild bird and swine feces. Applied and environmental microbiology 1994;**60(3)**, 1025
42. Sturegård, E., Hertervig, E., Sjunnesson, H. and Wadström, T. Helicobacter species in human colon biopsies. Alimentary pharmacology & therapeutics 2004;**19(5)**, 613-4
43. Takayama, S., Takahashi, H., Matsuo, Y., Okada, Y. and Takeyama H. Effect of Helicobacter bilis infection on human bile duct cancer cells. Digestive diseases and sciences 2010;**55(7)**, 1905-10
44. Taylor, L.H., Latham, S.M. and Mark, E. Risk factors for human disease emergence. Philosophical Transactions of the Royal Society of London Series B. Biological Sciences 2001;**356(1411)**, 983-9
45. Ward, J.M., Anver, M.R., Haines, D.C. and Benveniste, R.E. Chronic active hepatitis in mice caused by Helicobacter hepaticus. The American journal of pathology 1994;**145(4)**, 959
46. Warren, J.R. and Marshall, B. Unidentified curved bacilli on gastric epithelium in active chronic gastritis. The Lancet 1983;**321(8336)**, 1273-5
47. Whary, M.T., Cline, J.H., King, A.E., Hewes, K.M., Chojnacky, D., Salvarrey, A., et al. Monitoring sentinel mice for Helicobacter hepaticus, H. rodentium, and H. bilis infection by use of polymerase chain reaction analysis and serologic testing. Comparative Medicine 2000;**50(4)**, 436-43

9/6/2012