THE NATIONAL RESEARCH COUNCIL OF THAILAND
RESEARCH LIBRARY

REFERENCES

Adam R, Hinz E, Sithithaworn P, Pipitgool V, Storch V. Ultrastructural hepatic alterations in hamsters and jirds after experimental infection with the liver fluke *Opisthorchis viverrini*. **Parasitol Res** 1993; 79(5): 357-364.

Alpini G, Ueno Y, Glaser SS, Marzioni M, Phinizy JL, Francis H, Lesage G. Bile acid feeding increased proliferative activity and apical bile acid transporter expression in both small and large rat cholangiocytes. **Hepatology** 2001; 34: 868-876.

_____, Baiocchi L, Glaser S, Ueno Y, Marzioni M, Francis H, Phinizy JL, Angelico M, Lesage G. Ursodeoxycholate and tauroursodeoxycholate inhibit cholangiocyte growth and secretion of BDL rats through activation of PKC alpha. **Hepatology** 2002; 35: 1041-1052.

Apinhasmit W, Sobhon P, Saitongdee P, Upatham ES. *Opisthorchis viverrini*: changes of the tegumental surface in newly excysted juvenile, first-week and adult flukes. **Int J Parasitol** 1993; 23(7): 829-839.

Attili AF, Angelico M, Cantafora A, Alvaro D, Capocaccia L. Bile acid-induced liver toxicity: relation to the hydrophobic-hydrophilic balance of bile acids. **Med Hypo** 1986; 19: 57-69.

Bhamarapravati N, Thammavit W, Vajrasthira S. Liver changes in hamsters infected with a liver fluke of man, *Opisthorchis viverrini*. **Am J Trop Med Hyg** 1978; 27(4): 787-794.

Boonjaraspinyo S, Boonmars T, Aromdee C, Srisawangwong T, Kaewsamut B, Pinlaor S, Yongvanit P, Puapairoj A. Turmeric reduces inflammatory cells in hamster opisthorchiasis. **Parasitol Res** 2009; 105: 1459-1463.

_____, Boonmars T, Aromdee C, Puapairoj A, Wu Z. Indirect effect of a turmeric diet: enhanced bile duct proliferation in Syrian hamsters with a combination of partial obstruction by *Opisthorchis viverrini* infection and inflammation by N-nitrosodimethylamine administration. **Parasitol Res** 2011; 108(1): 7-14.

Boonmars T, Wu Z, Boonjaruspinyo S, Pinlaor S, Nagano I, Takahashi Y, Kaewsamut B, Yongvanit P. Alterations of gene expression of RB pathway in *Opisthorchis viverrini* infection-induced cholangiocarcinoma. **Parasitol Res** 2009; 105(5): 1273-1281.

_____, Boonjaraspinyo S, Kaewsamut B. Animal models for *Opisthorchis viverrini* infection. **Parasitol Res** 2009; 104: 701-703.

Boonpucknavig S, Boonpucknavig V, Tanvanich S, Doungchawee G, Thamavit W. Development of immune-complex glomerulonephritis and amyloidosis in Syrian golden hamsters infected with *Opisthorchis viverrini*. **J Med Assoc Thai** 1992; 75(1): 7-19.

Bychkov VG, Krylov GG, Sabirov AKh, Zuevskiĭ VP, Khadieva ED, Zhandekov AK. Hepatic cell transformation changes in experimental opisthorchiasis. **Med Parazitol (Mosk)** 2008; 3: 9-12.

Changbumrung S, Tungtrongchitr R, Migasena P, Chamreonngan S. Serum unconjugated primary and secondary bile acids in patients with cholangiocarcinoma and hepatocellular. **J Med Assoc Thai** 1990; 73(2): 81-89.

Deo Bandiera. Identification of human hepatic cytochrome P450 enzymes involved in the biotransformation of cholic and chenodeoxycholic acid. **Drug Metabilism and Disposition** 2008; 36(10): 1983-1991.

Dhiensiri T, Eua-Ananta Y, Bunnag D, Harinasuta T, Schelp PF. Roentgenographically controlled healing of gallbladder lesions in opisthorchiasis after praziquantel treatment. **Arzneimittel-forsch** 1984; 34: 1175-1177.

Egon Stahl. **Thin layer chromatography: A laboratory handbook**. 2nd Ed. New York: Springer-Verlag Berlin Heidelberg; 1969.

Einarsson C, Ellis E, Abrahamsson A, Ericzon Bo-Göran, Ingemar Björkhem, Axelson M. Bile acid formation in primary human hepatocytes. **World J Gastroentero** 2000; 6(4): 522-525.

Elkins DB, Haswell-Elkins MR, Mairiang E, Mairiang P, Sithithaworn P, Kaewkes S, Bhudhisawasdi V, Uttaravichien T. A high frequency of hepatobiliary disease and suspected cholangiocarcinoma associated with heavy *Opisthorchis viverrini* infection in a small community in north-east Thailand. **Trans R Soc Trop Med Hyg** 1990; 84: 715-719.

Elkins DB, Mairiang E, Sithithaworn P, Mairiang P, Chaiyakum J, Chamadol N, Loapaiboon V, Haswell-Elkins M.R. Cross-sectional patterns of hepatobiliary abnormalities and possible precursor conditions of cholangiocarcinoma associated with *Opisthorchis viverrini* infection in humans. **Am J Trop Med Hyg** 1996; 55: 295-301.

Flavell DJ. Acquired resistance to *Opisthorchis viverrini* in the hamster. **Trans R Soc Trop Med Hyg** 1982; 76(6): 800-802.

Flavell DJ, Flavell SU, Field GF. *Opisthorchis viverrini:* the relationship between egg production, worm size and intensity of infection in the hamster. **Trans R Soc Trop Med Hyg** 1983; 77(4): 538-545.

Fischer S, Beuers U, Spengler U, Zwiebel FM, Koebe HG. Hepatic levels of bile acids in end-stage chronic cholestatic liver disease. **Clin Chim Acta** 1996; 251: 173-186.

Ginovker AG, Zuevskiĭ VP. Ultrastructural analysis of the hepatocytes and hepatic bile duct epithelium of the golden hamster with experimental opisthorchiasis. **Med Parazitol (Mosk)** 1985; (5): 14-17.

_____, Bychkov VG, Solov'ev GS. Effect of an Opisthorchis infestation on implantation growth of the liver epithelium. **Biull Eksp Biol Med** 1985; 99(1): 99-102.

Glinshirt H, Koss FW, Morianz K. Studies on the quantitative evaluation of thin-layer chromatography: Separation and determination of bile acids. **Arzneimittel- forsch** 1960; 10: 943-947.

Glumov V. Peculiarities of parasitic cirrhosis of the liver of guinea pigs in experimental opisthorchiasis. **Med Parazitol (Mosk)** 1974; 43(3): 289-293.

Greim H, Trulzsch D, Czygan P, Rudick J, Hutterer F, Schaffner F, et al. Mechanism of cholestasis: bile acids in human livers with or without biliary obstruction. **Gastroenterology** 1972; 63: 846-850.

Guo R, Luo Z, Wang F, Zhu G. Changes in bile composition in rabbits infected with Clonorchis sinensis. **Zhongguo Ji Sheng Chong Xue Yu Ji Sheng Chong Bing Za Zhi** 1990; 8(1): 29-31.

Harinasuta C, Harinasuta T. *Opisthorchis viverrini*: life cycle, intermediate hosts, transmission to man and geographical distribution in Thailand. **Arzneimittel-forsch** 1984; 34(9B): 1164-1167.

Harinasuta T, Riganti M, Bunnag D. *Opisthorchis viverrini* infection: pathogenesis and clinical features. **Arzneimittel-forsch** 1984; 34(9B): 1167-1169.

Haswell-Elkins M.R, Sithithaworn P, Mairiang E, Elkins D.B, Wongratanacheewin S, Kaewkes S, Mairiang P. Immune responsiveness and parasite-specific antibody levels in human hepatobiliary disease associated with *Opisthorchis viverrini* infection. **Clin Exp Immunol** 1991; 84: 213-218.

Ilyinskikh EN, Lepyehin AV, Logvinov SV, Ilyinskikh NN. Estimation of the mutagenic potential of the trematode *Opisthorchis felineus* in experimentally infected guinea pigs. **Parasitol Res** 1998; 84: 570-572.

International Agency for Research on Cancer (IARC). Infection with liver flukes (*Opisthorchis viverrini, Opisthorchis felineus* and *Clonorchis sinensis*). **IARC Monogr Eval Carcinog Risks Hum** 1994; 61: 121-175.

Ishak K, Baptista A, Bianchi L, Callea F, De Groote J, Gudat F, et al. Histological grading and staging of chronic hepatitis. **J Hepatol** 1995; 22: 696-699.

Ito K, Muto M. **Byorigaku kiyo**. 1925; 15: 565-571. Cited by Komiya Y. Clonorchis and clonorchiasis. **Advance in Parasitology** 1966; 4: 53-106.

Iwata S. **Jikken Shokakibyo Grakkai Zasshi** 1937; 12: 1872-1886. Cited by Komiya Y. Clonorchis and clonorchiasis. **Advance in Parasitology** 1966; 4: 53-106.

Javitt NB. Bile acid synthesis from cholesterol: regulatory and auxiliary pathways. **FASEB J** 1994; 8: 1308-1311.

Jessica E Enes, Aaron J Wages, John B Malone, Smarn Tesana. Prevalence of *Opisthorchis verririni* infection in the canine and feline hosts three villages, Khon kaen province, Northeastern Thailand. **Southeast Asian J Trop Med Public Health** 2010; 41(1): 36-42.

John Y, Chiang L. Regulation of bile acids synthesis. **Front Biosci** 1998; 3: 176-193.

Jongsuksuntigul P, Chaychumsri W, Techamontrikul P, Cheeradit P, Suratawanich P. Studies on prevalence and intensity of intestinal helminthiasis and liver fluke in Thailand in 1991. Report of a national survey by the Helminth Division Ministryof Public Health, Thailand. **J Trop Med Assoc Thai** 1992; 2: 80-95.

_____, Imsomboon T. Opisthorchiasis control in Thailand. **Acta Trop** 2003; 88(3): 229-232.

Jongsuksuntigul P. Parasitic diseases in northeast Thailand. In: **Seminar in Parasitic Diseases in Northeast Thailand;** 2002 November 22–23; Khon Kaen. Khon Kaen; 2002. p. 3-18.

Kaewkes S, Elkins DB, Sithithaworn P, Haswell-Elkins MR. Comparative studies on the morphology of the eggs of *Opisthorchis viverrini* and lecithodendriid trematodes. Southeast Asian J Trop Med Public Health 1991; 22(4): 623-630.

_____.Taxonomy and biology of liver flukes. **Acta Trop** 2003; 88(3): 177-186.

Keiser J, Shu-Hua X, Jian X, Zhen-San C, Odermatt P, Tesana S, Tanner M, Utzinger J. Effect of artesunate and artemether against *Clonorchis sinensis* and *Opisthorchis viverrini* in rodent models. **Int J Antimicrob Agents** 2006; 28: 370-373.

Kim TI, Cho PY, Yoo WG, Li S, Hong SJ. Bile-induced genes in *Clonorchis sinensis* metacercariae. **Parasitol Res** 2008; 103: 1377-1382.

Komiya Y. Clonorchis and clonorchiasis. **Advance in Parasitology** 1966; 4: 53-106.

Koompirochana V, Sonakul S, Chinda K, Stitnimankarn T. Opisthorchiasis: a clinicopathologic study of 154 autopsy cases. **Southeast Asian J Trop Med Public Health** 1978; 9: 60-64.

Koulentaki M, Koutroubakis IE, Petinaki E, Tzardi M, Oekonomaki H, Mouzas I, Kouroumalis EA. Ulcerative colitis associated with primary biliary cirrhosis. **Dig Dis Sci** 1999; 44(10):1953-1956.

Knodell RG, Ishak KG, BlackWC,Chen TS, Craig R, Kaplowitz N, et al. Formulation and application of a numerical scoring system for assessing histological activity in asymptomatic chronic active hepatitis. **Hepatology** 1981; 1: 431-435.

Krishnamurthy GT and Krishnamurthy S. **Nuclear hepatology: a textbook of hepatobiliary diseases**. 1st Ed. Berlin: Springer; 2000. p. 291.

Lai CH, Chin C, Chung HC, Liu H, Hwang JC, Lin HH. Clonorchiasis-associated perforated eosinophilic cholecystitis. **Am J Trop Med Hyg** 2007; 76(2): 396-398.

Lai XZ, Feng Y, Pollard J, Chin JN, Rybak MJ, Bucki R, Epand RF, Epand RM, Savage PB. Ceragenins: cholic acid-based mimics of antimicrobial peptides. **Acc Chem Res** 2008; 41(10): 1233-1240.

Laurence M. Harwood, Christopher J. Moody. **Experimental organic chemistry: Principles and Practice**. Illustrated Ed. [n.p.]: p. 159-173.

Lapteva GF. Opisthorchiasis-related nephropathy. **Vrach Delo** 1990; 2: 67-69.

LeSage G, Glaśer Ş, Alpini G. Regulation of cholangiocyte proliferation. **Liver** 2001; 21: 73-80.

Loilom W, Juntana S, Namwat N, Bhudhisawasdi V, Puapairoj A, Sripa B, Miwa M, Saya H, Riggins GJ, Yongvanit P. PRKAR1A is overexpressed and represents a possible therapeutic target in human cholangiocarcinoma. **Int J Cancer** 2010; 7: DOI: 10.1002/ijc.25646.

Li S, Kim TI, Yoo WG, Cho PY, Kim TS, Hong SJ. Bile components and amino acids affect survival of the newly excysted juvenile *Clonorchis sinensis* in maintaining media. **Parasitol Res** 2008; 103: 1019-1024.

Lim JH. Radiologic findings of clonorchiasis. **AJR** 1990; 155: 1001-1008.

Mahler H, Christensen NO, Hindsbo O. Studies on the reproductive capacity of *Echinostoma caproni* (Trematoda) in hamsters and jirds. **Int J Parasitol** 1995; 25(6): 705-710.

Mairiang P, Bhudhisawasdi V, Borirakchanyavat V, Sitprija V. Acute renal failure in obstructive jaundice in cholangiocarcinoma. **Arch Intern Med** 1990; 150: 2357-2360.

Mairiang E, ElkinsD.B, Mairiang P, Chaiyakum J, Chamadol N, Loapaiboon V, Posri S, Sithithaworn P, Haswell-ElkinsMR. Relationship between intensity of *Opisthorchis viverrini* infection and hepatobiliary disease detected by ultrasonography. **J Gastroenterol Hepatol** 1992; 7: 17-21.

Mayer DA, Fried B. The role of helminth infections in carcinogenesis. **Adv Parasitol** 2007; 65: 239-296.

Melling N, Hohenberger W, Yedibela SL. Case report: Opisthorchiasis mimicking primary biliary cirrhosis as an indication for liver transplantation. **J Hepatology** 2009; 50: 1057-1059.

Nithikathkul C, Tesana S, Sithithaworn P, Balakanich S. Early stage biliary and intrahepatic migration of *Opisthorchis viverrini* in the golden hamster. **J Helminthol** 2007; 81(1): 39-41.

Noble, Elmer R. **Parasitology: the biology of animal parasites.** 6Th Ed. Philadelphia: Lea and Febiger; 1989.

Parkin DM, Ohshima H, Srivatanakul P, Vatanasapt V. Cholangiocarcinoma: epidemiology, mechanisms of carcinogenesis and prevention. **Cancer Epidemiol Biomarkers Prev** 1993; 2(6): 537-544.

Pinlaor S, Yongvanit P, Prakobwong S, Kaewsamut B, Khoontawad J, Pinlaor P, Hiraku Y. Curcumin reduces oxidative and nitrative DNA damage through balancing of oxidant-antioxidant status in hamsters infected with *Opisthorchis viverrini*. **Mol Nutr Food Res** 2009; 53(10): 1316-1328.

Prakobwong S, Yongvanit P, Hiraku Y, Pairojkul C, Sithithaworn P, Pinlaor P, Pinlaor S. Involvement of MMP-9 in peribiliary fibrosis and cholangiocarcinogenesis via Rac1-dependent DNA damage in a hamster model. **Int J Cancer** 2010; 127(11): 2576-2587.

Pungpak S, Bunnag D, Harinasuta T. Clinical and laboratory evaluation of praziquantel in opisthorchiasis. **Southeast Asian J Trop Med Public Health** 1983; 14(3): 363-366.

Rangsin R, Mungthin M, Taamasri P, Mongklon S, Aimpun P, Naaglor T, Leelayoova S. Incidence and risk factors of *Opisthorchis viverrini* infections in a rural community in Thailand. **Am J Trop Med Hyg** 2009; 81(1):152-155.

Rohela M, Johari S, Jamaiah I, Init I, Lee SH. Acute cholecystitis caused by *Clonorchis sinensis*. **Southeast Asian J Trop Med Public Health** 2006; 37(4): 648-651.

Riganti M, Pungpak S, Punpoowong B, Bunnag D, Harinasuta T. Human pathology of *Opisthorchis viverrini* infection: a comparison of adults and children. **Southeast Asian J Trop Med Public Health** 1989; 20: 95-100.

Rodrigues CM, Fan G, Wong PY, Kren BT, Steer CJ. Ursodeoxycholic acid may inhibit deoxycholic acid-induced apoptosis by modulating mitochondrial transmembrane potential and reactive oxygen species production. **Mol Med** 1998; 4: 165-178.

Saowakontha S, Pipitgool V, Pariyanonda S, Tesana S, Rojsathaporn K, Intarakhao C. Field trials in the control of *Opisthorchis viverrini* with an integrated programme in endemic areas of northeast Thailand. **Parasitology** 1993; 106: 283-288.

Savage PB, Li C. Cholic acid derivatives: novel antimicrobials. **Expert Opin Investig Drugs** 2000; 9(2): 263-272.

Schuster RK, Heidrich J, Pauly A, Nockler K. Liver flukes in dogs and treatment with praziquantel. **Vet Parasitol** 2007; 150: 362-365.

Setchell KD, Rodrigues CM, Clerici C, Solinas A, Morelli A, Gartung C, et al. Bile acid concentrations in human and rat liver tissue and in hepatocyte nuclei. **Gastroenterology** 1997; 112: 226-235.

Sithithaworn P, Tesana S, Pipitgoon V, Kaewkes S, Pairojkul C, Sripa B, Paupairoj A, Thaiklar K. Relationship between fecal egg count and worm burden of *Opisthorchis viverrini* in human autopsy cases. **Parasitology** 1991a; 102: 277-281.

_____, Haswell-Elkins M. Epidemiology of *Opisthorchis viverrini*. **Acta Trop** 2003; 88(3): 187-194.

Sokol RJ, Devereaux M, Khandwala R, O'Brien K. Evidence for involvement of oxygen free radicals in bile acid toxicity to isolated rat hepatocytes. **Hepatology** 1993; 17: 869-881.

Songserm N, Prasongwattana J, Sithithaworn P, Sripa B, Pipitkool V. Cholangiocarcinoma in experimental hamsters with long-standing *Opisthorchis viverrini* infection. **Asian Pac J Cancer Prev** 2009; 10(2): 299-302.

Sriamporn S, Pisani P, Pipitgool V, Suwanrungruang K, Kamsa-ard S, Parkin DM. Prevalence of *Opisthorchis viverrini* infection and incidence of cholangiocarcinoma in Khon Kaen, northeast Thailand. **Trop Med Int Health** 2004; 9: 588-594.

Sripa B, Kaewkes S. Localisation of parasite antigens and inflammatory responses in experimental opisthorchiasis. **Int J Parasitol** 2000a; 30: 735-740.

_____, Kaewkes.S. Gallbladder and extrahepatic bile duct changes in *Opisthorchis viverrini*-infected hamsters. **Acta Tropica** 2002; 83: 29-36.

_____, Haswell-Elkins MR, Sinawat P. Histological analysis of gallbladder diseases in relation to opisthorchiasis in endemic areas of Thailand. **Acta Tropica** 2003; 88: 239-246.

_____. Pathobiology of opisthorchiasis:an update. **Acta Trop** 2003; 88(3): 209-220.

_____, Kaewkes S, Sithithaworn P, Mairiang E, Laha T, Smout M, et al. Liver fluke induces cholangiocarcinoma. **PLoS Med** 2007; 4(7): 201.

Sripa B, Mairiang E, Thinkhamrop B, Laha T, Kaewkes S, Sithithaworn P, Tessana S, Loukas A, Brindley PJ, Bethony JM. Advanced periductal fibrosis from infection with the carcinogenic human liver fluke *Opisthorchis viverrini* correlates with elevated levels of interleukin-6. **Hepatology** 2009; 50(4): 1273-1281.

Sullivan WG, Koep LJ. Common bile duct obstruction and cholangiohepatitis in clonorchiasis. **JAMA** 1980; 243(20): 2060-2061.

Tansurat P. **Pathology of Protozoal and Helminthic Diseases.** Baltimore: Williams and Wilkins; 1971. p. 536-545.

Thin-layer chromatography. [cited 2010 Nov 12]. Available from http://www.chem.wisc.edu/courses/342/Fall2004/TLC.pdf

Upatham ES, Viyanant V, Kurathong S, Rojborwonwitaya J, Brockelman WY, Ardsungnoen S. Relationship between prevalence and intensity of *Opisthorchis viverrini* infection and clinical symptoms and signs in a rural community in northeast Thailand. **Bull World Health Organ** 1984; 62(3): 451-461.

_____, Viyanant V, Brockelman WY, Kurathong S, Lee P, Kraengraeng R. Rate of re-infection by *Opisthorchis viverrini* in an endemic northeast Thai community after chemotherapy. **Int J Parasitol** 1988; 18: 643-669.

Vajrasthira S, Harinasuta C, Komiya Y. The morphology of the metacercaria of *Opisthorchis viverrini*, with special reference to the excretory system. **Ann Trop Med Parasitol** 1961; 55: 413-418.

Viranuvatti V, Stitnimankarn T. Liver fluke infection and infestation in Southeast Asia. **Prog Liver Dis** 1972; 4: 537-547.

Waikagul J. *Opisthorchis viverrini* metacercaria in Thai freshwater fish. **Southeast Asian J Trop Med Public Health** 1998; 29(2): 324-326.

Wannaprapo S, Tesana S, Arunyanat C, Kanla P, Chaijaroonkhanarak W. Morphological Features of the Testes of the Adult Liver Fluke, *Opisthorchis viverrini.* **Srinagarind Med J** 2008; 23(4): 346-352.

Wongpaitoon V, Thuvasethakul P, Kurathong S, Techanugorn S. Biliary bile acids and lipids composition in *Opisthorchiasis viverrini*. **J Med Assoc Thai** 1988; 71(7): 394-399.

Wonkchalee O, Boonmars T, Kaewkes S, Chamgramol Y, Pairojkul C, Wu Z, Juasook A, Sudsarn P, Boonjaraspinyo S. *Opisthorchis viverrini* infection causes liver and biliary cirrhosis in gerbils. DOI 10.1007/s00436-011-2282-y.

World Health Organization. **Control of foodborne trematode infections**. Geneva: WHO; 1995. p. 69-70.

Wykoff DE, Ariyaprakul K. *Opisthirchis viverrini* in Thailand, egg production in man and laboratory animals. **The Journal of Parasitology** 1966; 53: 631.

Xiao WB, Liu YL. Primary biliary cirrhosis and ulcerative colitis: a case report and review of literature. **World J Gastroenterol** 2003; 9(4):878-880.

Yerushalmi B, Dahl R, Devereaux MW, Gumpricht E, Sokol RJ. Bile acid-induced rat hepatocyte apoptosis is inhibited by antioxidants and blockers of the mitochondrial permeability transition. **Hepatology** 2001; 33: 616-626.

Zimber A, Gespach C. Bile acids and derivatives, their nuclear receptors FXR, PXR and ligands: role in health and disease and their therapeutic potential. **Anti Canc Agents Med Chem** 2008; 8: 5540-5563.

APPENDICES

APPENDIX A

Solution preparation for metacercariae

1. 0.25% Pepsin solution

Chemical reagents

- Pepsin powder	2.5	g
- Concentration hydrochloric acids	1.0	ml
- Distilled water	1000	ml

2. 0.85% NaCl

Chemical reagents

- NaCl	8.5	g
- Distilled water	1000	ml

APPENDIX B

Solution preparation for eggs per gram

1. **1% Iodine solution**

Chemical reagents

- Potassium iodide 50 g
- Iodine 70 g
- Distilled water 50 ml

2. **95% ethyl alcohol**

Chemical reagents

- Absolute alcohol 95 ml
- Distilled water 5 ml

Dissolve 50 g of potassium iodide in 50 mL of DI water; add 70 g iodine; stir to dissolve then dilute to 1 L with 95% ethyl alcohol. Store in a dark bottle.

3. **Formalin Solution (10%, unbuffered)**

Chemical reagents

- Formaldehyde (37-40) 10 ml
- Distilled water 90 ml

APPENDIX C

Solution preparation for eggs per worm

1. 70% ethyl alcohol

Chemical reagents

- Absolute alcohol	70	ml
- Distilled water	30	ml

THE NATIONAL RESEARCH COUNCIL OF THAILAND RESEARCH LIBRARY

APPENDIX D

Solution preparation for carmine staining

1. **Preparation of Carmine**

Chemical reagents

- Carmine	25	g
- Glacial acetic acids	25	ml
- Salicylic acids	1	g
- Aluminum potassium sulfate	25	mg
- Distilled water	1000	ml

The method of preparation

- Crushed Carmine thoroughtly with mortar.
- Dissolved Carmine in 10 ml of distilled water and then add 25 ml of glacial acetic acids after that, leave it set 25 minutes.
- Boiling for 1 hr then leave it set to cool.
- Dissolved 25 g. of Aluminum potassium sulfate in 500 ml of distilledwater and then was added to cooled Carmine solution.
- Boiling for 1 hr then leave it set to cool after that filtered through filter paper.
- Add 1 g. of Salicylic acids was stir well and store in gloss bottles.

2. **70% ethyl alcohol**

Chemical reagents

- Absolute alcohol	70	ml
- Distilled water	30	ml

3. **80% ethyl alcohol**

Chemical reagents

- Absolute alcohol	80	ml
- Distilled water	20	ml

4. 90% ethyl alcohol

Chemical reagents

- Absolute alcohol	90	ml
- Distilled water	10	ml

5. 95% ethyl alcohol

Chemical reagents

- Absolute alcohol	95	ml
- Distilled water	5	ml

6. 1% acid-alcohol

Chemical reagents

- Cocentrated Hydrochloric acid	5	ml
- Ethyl alcohol	495	ml

APPENDIX E

Solution preparation for thin-layer chromatography

1. Cholic acid standard

Chemical reagents

- Cholic acid	1	mg
- Methanol	1	ml

2. Chenodeoxycholic acid

Chemical reagents

- Chenodeoxycholic acid	1	mg
- Methanol	1	ml

3. 2% w/v vanilline

Chemical reagents

- Vanilline	0.2	g
- Methanol	10	ml

THE NATIONAL RESEARCH COUNCIL OF THAILAND RESEARCH LIBRARY

4. 10% v/v sulphuric acids

Chemical reagents

- Sulphuric acids	1	ml
- Methanol	9	ml

5. Mobile phase of mixture

Chemical reagents

- Hexane	7	ml
- Ethyl acetate	23	ml
- Glacial acetic acid	3	ml
- Methanol	2	ml

APPENDIX F

Solution preparation for hematoxylin and eosin staining

1. Hematoxylin and eosin staining

1.1 Fizative liver section

Chemical reagents

- 100% Formaldehyde	100	ml
- Distilled water	5	ml

1.2 Harris's hematoxylin

Chemical reagents

- Hematoxylin crystal	5	g
- Absolute alcohol	50	ml
- Ammonium or potassium alum	100	ml
- Distilled water	100	ml
- Mercuric oxide	2.5	g

1.3 1% stock alcohol eosin

Chemical reagents

- Eosin stock solution	1	part
- 80% alcohol	3	parts

Add glacial acetic acids 0.5 ml/ Eosin 100 ml before used.

APPENDIX G

Solution preparation for gomori's trichome staining

1. Bouin's Solution

Chemical reagents

- Picric acid, saturated aqueous	75.0	ml
- Formalin, concentrated, 37-40%	25.0	ml
- Acetic acid, glacial	5.0	ml

2. Modified Weigert's Iron Hematoxylin

2.1 Solution A

Chemical reagents

- Hematoxylin crystals, C.I. 75290	2.0	g
- 90% ethyl alcohol	100.0	ml

2.2 Solution B

Chemical reagents

- Ferric chloride, FeCl3, 6H20, 62% aqueous	4.0	ml
- Distilled water	95.0	ml
- Hydrochloric acid, concentrated	1.0	ml

For use mix equal parts of Solution A and Solution B.

3. Gomori's trichrome Stain

Chemical reagents

- Chromotrope 2R, C.I. 16570	0.6	g
- Aniline blue, C.I. 42755	0.3	g
- Acetic acid, glacial	1	ml
- Phosphotungstic acid	0.8	g
- Distilled water	100	ml

4. 0.5% Acetic Acid

Chemical reagents

- Acetic acid, glacial	0.5	ml
- Distilled water	99.5	ml

5. 0.5% hydrochloric acid in 70% alcohol

Chemical reagents

- Hydrochloric acid, concentrated	0.5	ml
- 70% ethyl alcohol	99.5	ml

APPENDIX H

Tables

Table 10 Eggs per gram of feces in infected hamsters and gerbils at 30, 60 and 90 days post-infection.

Group		N	Mean ± SEM	Min - Max
Hamsters	30 Dpi	10	1927.78 ± 245.11	829.6 - 3143.8
	60 Dpi	10	2588.36 ± 291.98	1462.8 - 4426.2
	90 Dpi	8	3349.43 ± 520.6	1459.9 - 5520.8
Gerbils	30 Dpi	10	3471.68 ± 519.92	981 - 6219.3
	60 Dpi	9	4947.47 ± 926.89	2061.9 - 10229.
	90 Dpi	9	7616.31 ± 1635.67	596.7 - 15057.

Table 11 The eggs from collected *O.viverrini* adult uterus were crushed at 30, 60 and 90 days post-infection compared between infected hamsters and gerbils.

Group		n	Mean ± SEM	Min - Max
Hamsters	30 Dpi	5	4278.9 ± 602.5	2635.3 - 5950
	60 Dpi	5	8397.1 ± 1661.9	2238.7 - 11993.3
	90 Dpi	5	13505.9 ± 1271.6	11136.7 - 18368
Gerbils	30 Dpi	5	7220.5 ± 848.2	7320 - 10206
	60 Dpi	5	46842.0 ± 4547.6	37480 - 63080
	90 Dpi	5	55447.2 ± 3897.8	43296 - 66160

Table 12 Worm recovery of *O.viverrini* which was collected from infected hamsters and gerbils at 30, 60 and 90 days post-infection.

Group		n	Mean ± SEM	Min - Max
Hamsters	30 Dpi	10	27.9 ± 3.3	18 - 32
	60 Dpi	10	29.9 ± 1.5	19 - 35
	90 Dpi	8	30.4 ± 2.9	12 - 40
Gerbils	30 Dpi	10	13.1 ± 1.7	5 - 25
	60 Dpi	9	5 ± 1.2	1 - 12
	90 Dpi	9	6.4 ± 1.4	1 - 12

Table 13 Eggs per gram of feces per worm recovery of *O.viverrini* which was collected from infected hamsters and gerbils at 30, 60 and 90 days post-infection.

Group		Mean ± SEM	Min - Max
Hamsters	30 Dpi	45.4 ± 4.8	39 - 54.8
	60 Dpi	104.4 ± 13.6	63.4 - 147.5
	90 Dpi	109.3 ± 25.5	47.1 - 172.5
Gerbils	30 Dpi	208.4 ± 49.7	70.1 - 352.1
	60 Dpi	1876.1 ± 807	568.3 - 4933.1
	90 Dpi	1487.1 ± 853.1	298.36 - 4822.9

Table 14 Comparative mid-body width adult of *O.viverrini* adult which was collected from infected hamsters and gerbils at 30, 60 and 90 days post-infection.

Group		N	Mean ± SEM	Min - Max
Hamsters	30 Dpi	12	695.9 ± 36.6	453.07 - 911.29
	60 Dpi	15	1017.5 ± 34.8	856.12 - 1215.93
	90 Dpi	13	1048.2 ± 28.2	856.87 - 1167.97
Gerbils	30 Dpi	8	918.5 ± 53.2	745.4 - 1205.1
	60 Dpi	8	1335.4 ± 109.8	708.3 - 1704.4
	90 Dpi	6	1240.0 ± 41.0	1135.7 - 1387.84

THE NATIONAL RESEARCH COUNCIL OF THAILAND
RESEARCH LIBRARY

Table15 Comparative body length of *O.viverrini* adult which was collected from infected hamsters and gerbils at 30, 60 and 90 days post-infection.

Group		n	Mean ± SEM	Min - Max
Hamsters	30 Dpi	12	4031.7 ± 209.0	3364.85 - 4648.77
	60 Dpi	15	5006.5 ± 103.2	4386.66 - 5575.88
	90 Dpi	13	5425.1 ± 215.7	3514.37 - 6169.98
Gerbils	30 Dpi	8	4598.7 ± 175.1	3885.5 - 5420.0
	60 Dpi	8	5936.2 ± 323.2	5036.24 - 7875.4
	90 Dpi	6	6839.8 ± 388.0	5559.64 - 8204.79

Table 16 Comparative oral sucker width of *O.viverrini* adult which were collected from infected hamsters and gerbils at 30, 60 and 90 days post-infection.

Group		n	Mean ± SEM	Min - Max
Hamsters	30 Dpi	12	126.5 ± 5.7	94.69 - 149.6
	60 Dpi	15	156.2 ± 4.7	132 - 189.05
	90 Dpi	13	140.8 ± 6.8	99.39 - 174.45
Gerbils	30 Dpi	8	152.4 ± 8.7	106.5 - 188.8
	60 Dpi	8	132.3 ± 14.5	90.8 - 210.7
	90 Dpi	6	160.1 ± 6.0	144.63 - 180.24

Table 17 Comparative oral sucker length of *O.viverrini* adult which were collected from infected hamsters and gerbils at 30, 60 and 90 days post-infection.

Group		n	Mean ± SEM	Min - Max
Hamsters	30 Dpi	12	113.7 ± 4.6	85.04 - 135.86
	60 Dpi	15	152.0 ± 6.3	100.39 - 179.83
	90 Dpi	13	113.2 ± 8.7	62.12 - 167.08
Gerbils	30 Dpi	8	133.6 ± 9.9	101.8 - 180.3
	60 Dpi	8	60.7 ± 11.6	29.72 - 125.6
	90 Dpi	6	114.0 ± 12.0	69.11 - 151.5

Table 18 Comparative ventral sucker width of *O.viverrini* adult which were collected from infected hamsters and gerbils at 30, 60, 90 days post-infection.

Group		n	Mean ±	SEM	Min -	Max
Hamsters	30 Dpi	12	156.9 ±	4.5	132.83 -	174.83
	60 Dpi	15	179.6 ±	7.3	112.24 -	233.1
	90 Dpi	6	196.5 ±	17	131.53 -	253.55
Gerbils	30 Dpi	8	168.3 ±	8.4	131.9 -	214.4
	60 Dpi	8	187.6 ±	13.9	143 -	243.9
	90 Dpi	6	196.5 ±	17	131.53 -	253.55

Table 19 Comparative ventral sucker length of *O.viverrini* adult which were collected from infected hamsters and gerbils at 30, 60 and 90 days post-infection.

Group		n	Mean ±	SEM	Min -	Max
Hamsters	30 Dpi	12	152.1 ±	4.7	126.98 -	184.86
	60 Dpi	15	172.3 ±	5.9	133.86 -	204.61
	90 Dpi	13	172.2 ±	10.2	69.3 -	217.45
Gerbils	30 Dpi	8	155.8 ±	7.2	123.3 -	193.4
	60 Dpi	8	179.7 ±	13.1	126.6 -	203.5
	90 Dpi	6	182.2 ±	20.0	92.94 -	230.23

Table 20 Comparative ovary area of *O. viverrini* adult which were collected from infected hamsters and gerbils at 30, 60 and 90 days post-infection.

Group		n	Mean ± SEM	Min - Max
Hamsters	30 Dpi	12	24402.0 ± 3740	14086.71 - 54528.02
	60 Dpi	15	39326.4 ± 2914	12465.86 - 50735.19
	90 Dpi	13	34122.6 ± 3314.1	17960.09 - 53512.11
Gerbils	30 Dpi	8	54915.5 ± 5974	26676.8 - 78192.1
	60 Dpi	8	68388.4 ± 7324.4	28939.2 - 91804.64
	90 Dpi	6	104595.6 ± 15602	54024.72 - 143207.7

Table21 Comparative anterior testis area of *O.viverrini* adult which were collected from infected hamsters and gerbils at 30, 60 and 90 days post-infection.

Group		n	Mean ± SEM	Min - Max
Hamsters	30 Dpi	12	52253.7 ± 96.5	15640.51 - 56092.5
	60 Dpi	15	54512.9 ± 3796	17047.69 - 32151.16
	90 Dpi	13	65198.7 ± 5862.6	32441.4 - 94483.85
Gerbils	30 Dpi	8	114726.1 ± 10480.0	77394 - 185300.2
	60 Dpi	8	133480.7 ± 24346.2	44367.84 - 243704.16
	90 Dpi	6	248622.2 ± 39106	133889.8 - 382303.1

Table 22 Comparative posterior testis area of *O.viverrini* adult which were collected from infected hamsters and gerbils at 30, 60 and 90 days post-infection.

Group		n	Mean ± SEM	Min - Max
Hamsters	30 Dpi	12	54330.1 ± 9844	26444.99 - 150607.46
	60 Dpi	15	62408.8 ± 5565	32056.5 - 106174.19
	90 Dpi	13	71209.2 ± 6981.5	36810.26 - 103802.94
Gerbils	30 Dpi	8	123123.9 ± 12096.9	67316.8 - 1490002.5
	60 Dpi	8	141065.4 ± 26867.3	52610.1 - 256532.96
	90 Dpi	6	261034.3 ± 43097	163286.97 - 445924.16

Table 23 Comparative oral-ventral sucker distance of *O.viverrini* adult which were collected from infected hamsters and gerbils at 30, 60 and 90 days post-infection.

Group		n	Mean ± SEM	Min - Max
Hamsters	30 Dpi	12	957.9 ± 44.4	732.01 - 1295.24
	60 Dpi	15	1181.1 ± 73.2	531.42 - 1540.89
	90 Dpi	13	1355.3 ± 46.7	957.63 - 1581.22
Gerbils	30 Dpi	8	929.8 ± 46.2	739.6 - 1163.03
	60 Dpi	8	1066.2 ± 141.9	175.96 - 1476.24
	90 Dpi	6	1475.9 ± 56	1261.54 - 1643.07

Table 24 Comparative ovary-testes distance of *O.viverrini* adult which were collected from infected hamsters and gerbils at 30, 60 and 90 days post-infection.

Group		n	Mean ± SEM	Min - Max
Hamsters	30 Dpi	12	188.1 ± 29.6	56.67 - 323.65
	60 Dpi	15	184.4 ± 23.75	32.43 - 309.35
	90 Dpi	13	232.1 ± 28.5	82.78 - 463.58
Gerbils	30 Dpi	8	197.0 ± 16.9	116.16 - 309.7
	60 Dpi	8	223.2 ± 19.9	101.08 - 307.08
	90 Dpi	6	401.0 ± 17	41.88 - 142.55

Table 25 Comparative testes-testes distance of *O.viverrini* adult which were collected from infected hamsters and gerbils at 30, 60 and 90 days post-infection.

Group		n	Mean ± SEM	Min - Max
Hamsters	30 Dpi	12	49.4 ± 13.1	10 - 163.36
	60 Dpi	15	98.0 ± 13.64	24.88 - 205.08
	90 Dpi	13	102.3 ± 15.4	41.1 - 202.85
Gerbils	30 Dpi	8	34.8 ± 10.7	10 - 106.3
	60 Dpi	8	57.0 ± 30.7	4 - 130.12
	90 Dpi	6	34.9 ± 10	13.05 - 80.41

Table 26 Comparative testes-excretory pore distance of *O.viverrini* adult which were collected from infected hamsters and gerbils at 30, 60 and 90 days post-infection.

Group		n	Mean ± SEM	Min - Max
Hamsters	30 Dpi	12	274.2 ± 20.1	157.7 - 373.02
	60 Dpi	15	403.2 ± 19.98	260.57 - 556.19
	90 Dpi	13	439.5 ± 28.3	216.79 - 575.71
Gerbils	30 Dpi	8	297.8 ± 24.3	180 - 385.4
	60 Dpi	8	392.8 ± 53.1	231.2 - 621.92
	90 Dpi	6	321.0 ± 30	237.52 - 402.87

Table 27 Comparative alanine transminase level in uninfected normal control and infected hamsters and gerbils at 30, 60 and 90 days post-infection.

Group		n	Mean ± SEM	Min - Max
Hamsters	normal	3	109 ± 35.64	68 - 180
	30 Dpi	5	474.4 ± 124.39	24 - 752
	60 Dpi	5	417.8 ± 96.34	152 - 690
	90 Dpi	5	279.4 ± 59.49	113 - 485
Gerbils	normal	2	52.5 ± 24.5	28 - 77
	30 Dpi	4	995 ± 121	680 - 1260
	60 Dpi	4	245 ± 111.47	96 - 568
	90 Dpi	4	464.2 ± 25.06	295 - 416

Table 28 Comparative serum alkaline phosphatase level in uninfected normal control and infected hamsters and gerbils at 60 and 90 days post-infection.

Group		n	Mean ± SEM	Min - Max
Hamsters	normal	3	45.67 ± 5.51	40 - 51
	60 Dpi	5	77.8 ± 10.35	62 - 88
	90 Dpi	5	74.8 ± 17.52	58 - 99
Gerbils	normal	2	77 ± 4	73 - 81
	60 Dpi	4	150.25 ± 22.12	105 - 203
	90 Dpi	4	144.5 ± 20.22	94 - 181

RESEARCH PUBLICATION

Publication

1. **Orasa Wonkchalee,** Thidarut Boonmars, Sasithron Kaewkes, Yaovaluk Chamgramol, Chawalit Pairojkul, Zhiliang Wu, Amornrat Juasook, Pakkayanee Sudsarn, Sirintip Boonjaraspinyo. *Opisthorchis viverrini* infection causes liver and biliary cirrhosis in gerbils (Parasitology Research: DOI 10.1007/s00436-011-2282-y.)

Presentation

1. **Orasa Wonkchalee,** Sasithron Kaewkes, Yaovaluk Chamgramol , Amornrat Juasook, Pakkayanee Sudsarn , Sirintip Boonjaruspinyo , Thidarut Boonmars. Opisthirchiasis cause biliary cirrhosis in gerbil at Pre-congress Symposium, "Biomedical Sciences : Research for Healthy Society" Faculty of Medicine, Khon Kaen University on 11 October 2010: Poster presentation.
2. **Orasa Wonkchalee,** Thidarut Boonmars, Sasithron Kaewkes, Yaovaluk Chamgramol, Chawalit Pairojkul. *Opisthorchis viverrini* infections in hamster and gerbil: morphology and pathology at The 12th Graduate Research Conference 2011, Khon Kaen University on 28 January 2011: Poster presentation.

VITAE

THE NATIONAL RESEARCH COUNCIL OF THAILAND
RESEARCH LIBRARY

Name: Miss Orasa Wonkchalee

Date of Birth: October 27, 1985

Place of Birth: Nakhon Phanom, Thailand

Education:

2005-2009 Bachelor Degree of Nursing, Faculty of Nursing, Khon Kaen University, Khon Kaen, Thailand.

2009-2010 Graduated student in the major of Parasitology Program, Department of Parasitology, Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand.

Scholarships:

2009 A Grant of Graduate School and The Invitation Research Fund from Faculty of Medicine, Khon Kaen University

2010 Teacher assistance scholarship for Graduated student in Department of Parasitology, Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand.