CHAPTER IV
RESULTS

4.1 Parasite development through eggs per gram of feces

The parasite development was determined through eggs per gram of feces (Figure 12). The mean fecal egg counts of infected gerbils were more than those infected hamsters by increasing with the time manner of infection. At day 30 of infection, the fecal egg counts in all groups were not significant difference. Increased 2 folds of eggs in feces were observed at day 90 of infection. The eggs per gram of feces of i) infected hamsters at day 30 of infection, ii) infected hamsters at day 60 of infection, iii) infected hamsters at day 90 of infection, iv) infected gerbils at day 30 of infection, v) infected gerbils at day 60 of infection, vi) infected gerbils at day 90 of infection was followed 1927.78±245.11, 2588.36±291.98, 3349.43±520.6, 3471.68±519.92, 4947.47±926.89 and 7616.31±1635.6, respectively.

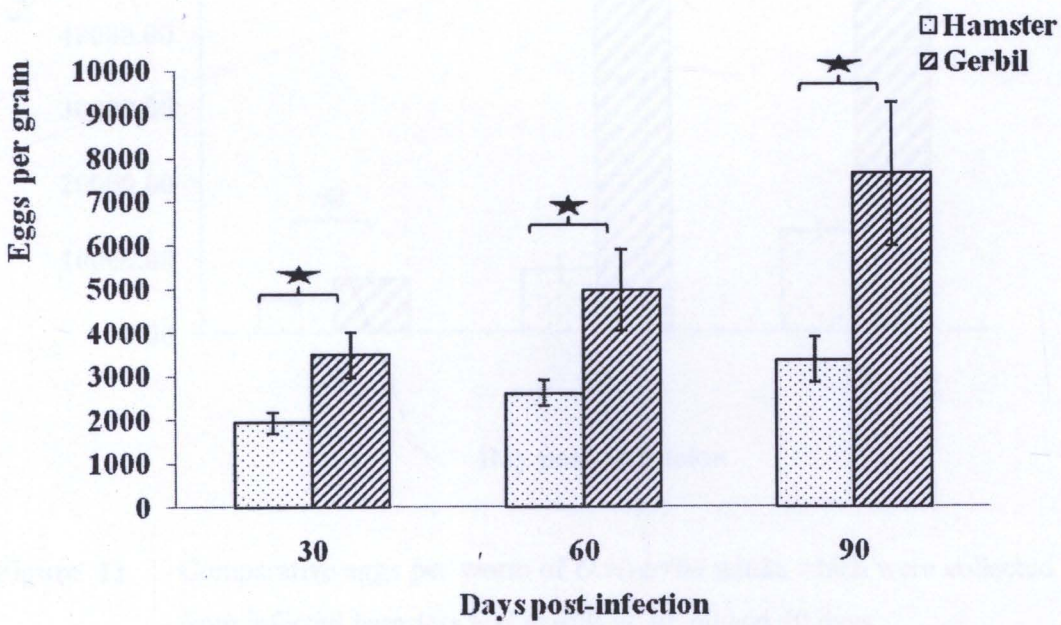

Figure 12 Comparative eggs per gram of feces which were collected from infected hamsters and gerbils at 30, 60 and 90 days post-infection.
★ Indicates a significant difference within group ($P<0.05$).

4.2 Parasite development through eggs per worm

The parasite development was determined through eggs per worm (Figure 13). The eggs per worm from all worms, which were collected from infected gerbils was more than all worms, which were collected from infected hamsters by increased with the time manner of infection. At day 30 of infection, the worm eggs in all groups were significant difference. Increased 6-7 folds of the worm eggs were observed at day 90 of infection. The worm eggs of i) infected hamsters at day 30 of infection, ii) infected hamsters at day 60 of infection, iii) infected hamsters at day 90 of infection, iv) infected gerbils at day 30 of infection, v) infected gerbils at day 60 of infection, vi) infected gerbils at day 90 of infection were 4278.9±602.5, 8397.1±1661.9, 13505.9±1271.6, 7220.5±848.2, 46842±4547.6 and 55447.2±3897.8 respectively.

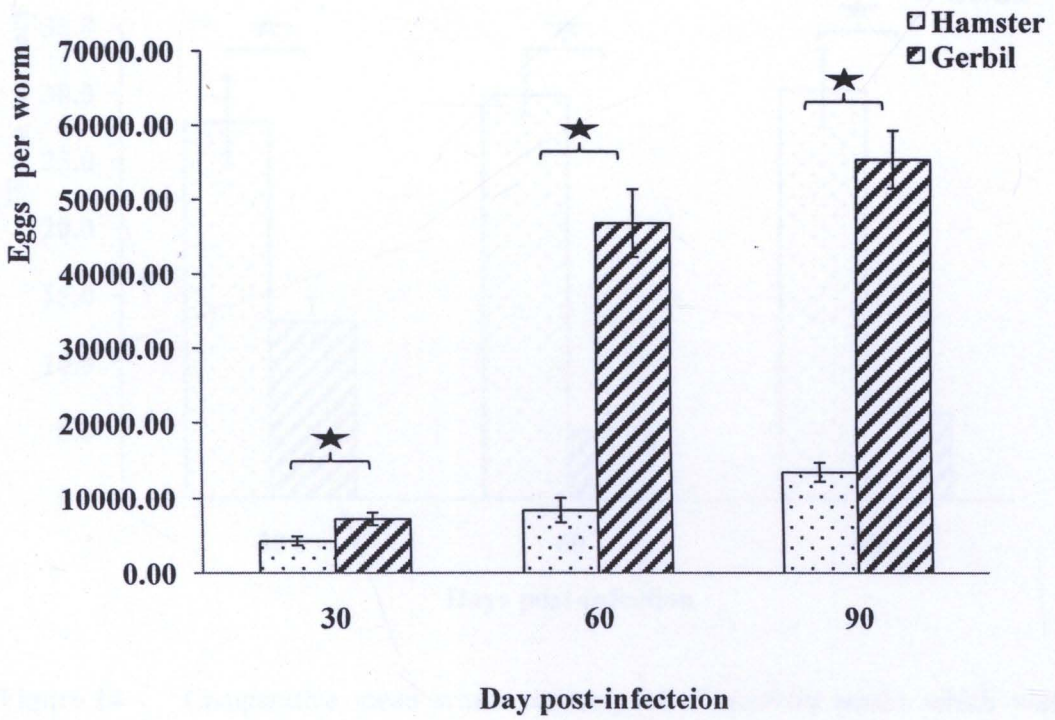

Figure 13 Comparative eggs per worm of *O.viverrini* adults which were collected from infected hamsters and gerbils at 30, 60 and 90 days post-infection.

★ Indicates a significant difference within group ($P<0.05$).

4.3 Worm recovery

Figure 14 showed the intensity of *O.viverrini* from each infected hamsters and gerbils. The worm recovery of all infected gerbils was less than form those infected hamsters by 2-5 folds changes. The worm recovery of i) infected hamsters at day 30 of infection, ii) infected hamsters at day 60 of infection, iii) infected hamsters at day 90 of infection, iv) infected gerbils at day 30 of infection, v) infected gerbils at day 60 of infection, vi) infected gerbils at day 90 of infection was followed 27.9±3.3, 29.9±1.5, 30.4±2.9, 13.1±1.7, 5±1.2 and 6.4±1.4 respectively.

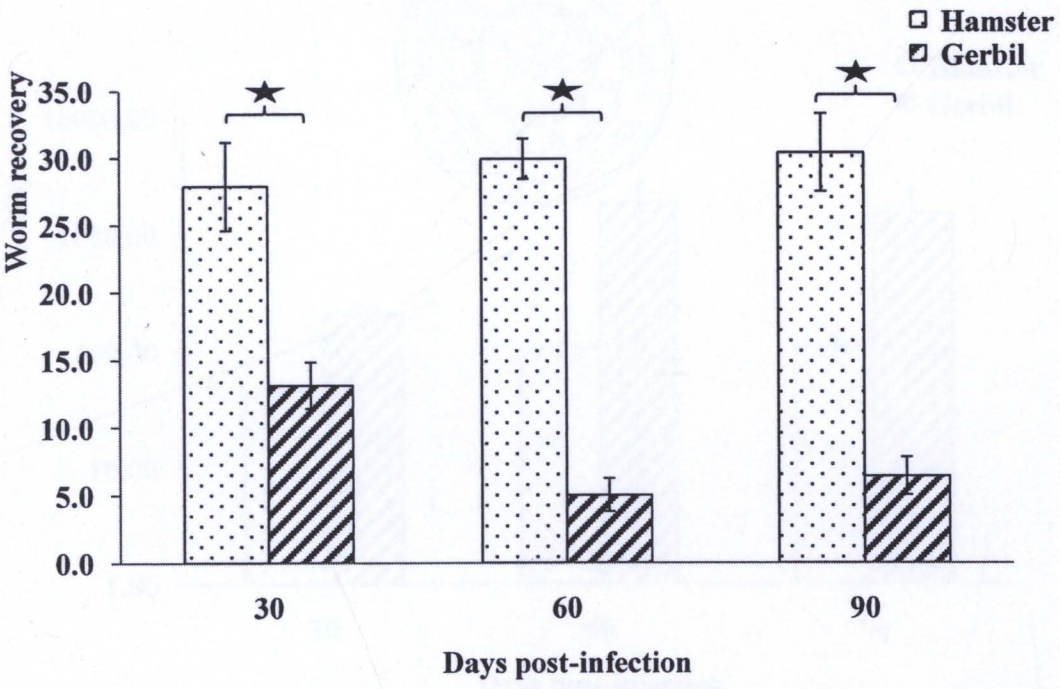

Figure 14 Comparative mean worm recovery of *O.viverrini* adults which was collected from infected hamsters and gerbils at 30, 60 and 90 days post-infection.

★ Indicates a significant difference within group ($P<0.05$).

4.4 Eggs per gram of feces per worm

The amount of eggs per gram of feces per worm of parasite was not significant difference. The both group tend to increase with time manner. The portion of eggs per gram of feces per worm in the infected gerbils was increased 5-18 fold than infected hamsters. The eggs per gram of feces per worm of i) infected hamsters at day 30 of infection, ii) infected hamsters at day 60 of infection, iii) infected hamsters at day 90 of infection, iv) infected gerbils at day 30 of infection, v) infected gerbils at day 60 of infection, vi) infected gerbils at day 90 of infection were 45.4±4.8, 104.4±13.6, 109.3±25.5, 208.4±49.7, 1876.1±807 and 1487.1±853.1 respectively.

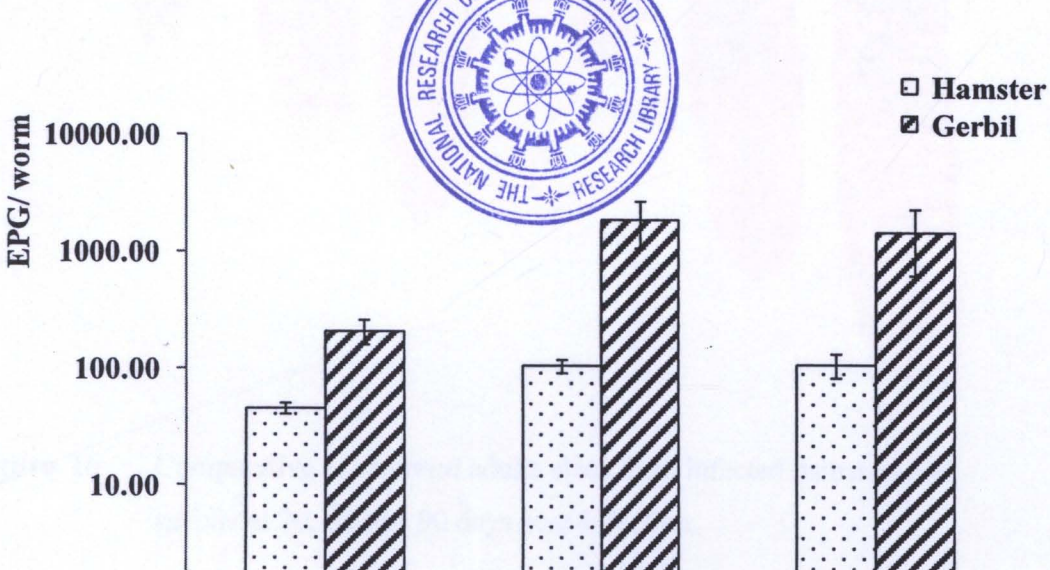

Figure 15 Eggs per gram of feces per worm of *O.viverrini* adults which was collected from infected hamsters and gerbils at 30, 60 and 90 days post-infection.

4.1 Body size of *O. viverrini* adult worm

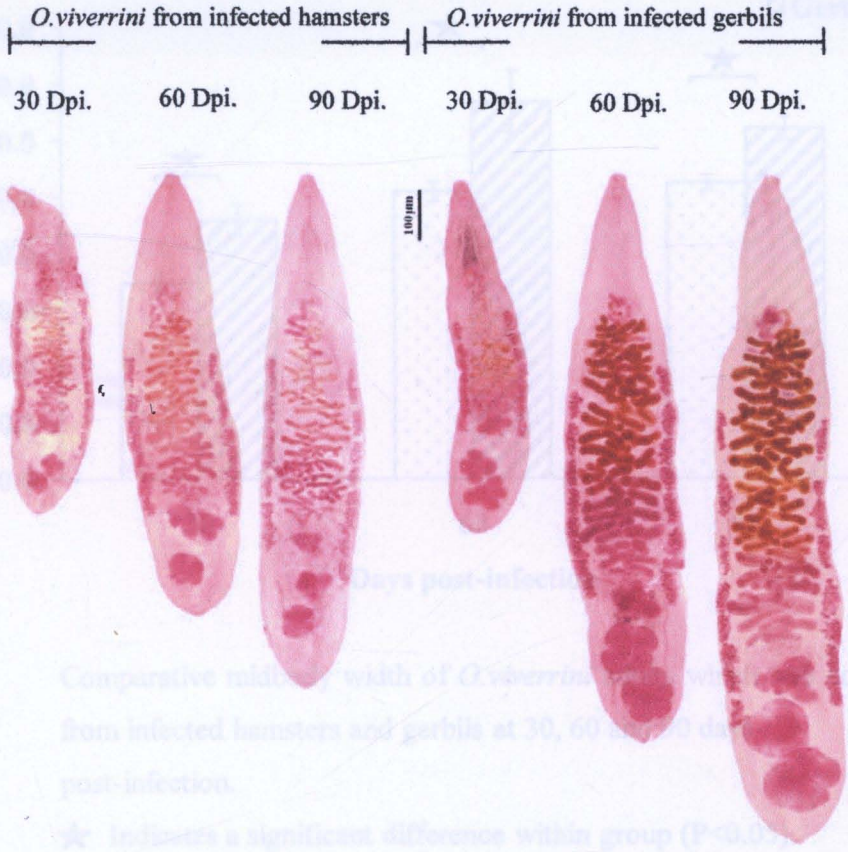

Figure 16 Comparative *O.viverrini* adults sizes from infected hamsters and gerbils at 30, 60 and 90 days post-infection.

4.5.1 The midbody width

The size of *O.viverrini* adult during development was determined from stained parasites under microscopic using image analysis. The body size of *O.viverrini* parasite was gradually increased in midbody width from day 30 to 90 (Figure 16). The midbody width of *O.viverrini* from infected gerbils was larger than infected hamsters (Figure 17). The width of *O.viverrini* i) infected hamsters at day 30 of infection, ii) infected hamsters at day 60 of infection, iii) infected hamsters at day 90 of infection, iv) infected gerbils at day 30 of infection, v) infected gerbils at day 60 of infection, vi) infected gerbils at day 90 of infection was 695.9±36.6, 1017.5±34.8, 1048.2±28.2, 918.5±53.2, 1335.4±109.8, and 1240±41 respectively.

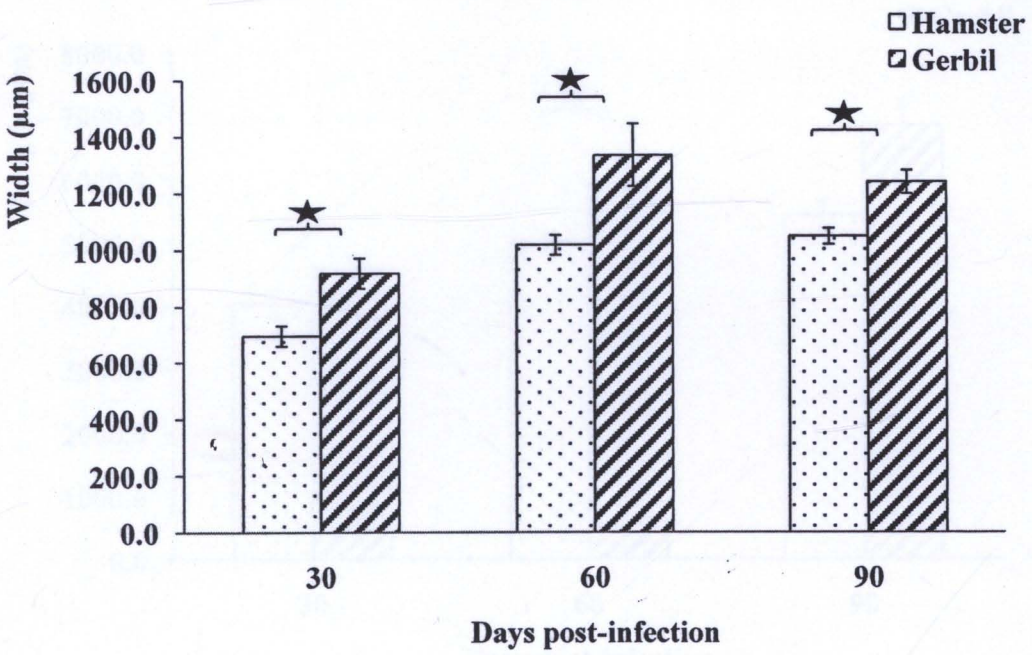

Figure 17 Comparative midbody width of *O.viverrini* adults which was collected from infected hamsters and gerbils at 30, 60 and 90 days post-infection.

★ Indicates a significant difference within group ($P<0.05$).

4.5.2 The body length

The body length of *O.viverrini* parasite was determined from stained worm using image analysis. The body length of *O.viverrini* increased in body length from day 30 of infection to day 90 of infection (Figure 16). The body length of worms which were collected from infected gerbils was longer than those worms from infected hamsters (Figure 18). The body length of *O.viverrini* i) infected hamsters at day 30 of infection, ii) infected hamsters at day 60 of infection, iii) infected hamsters at day 90 of infection, iv) infected gerbils at day 30 of infection, v) infected gerbils at day 60 of infection, vi) infected gerbils at day 90 of infection was 4031.7±209, 5006.5±103.2, 5425.1±215.7 4598.7±175.1, 5936.2±323.2 and 6839.8±388 respectively.

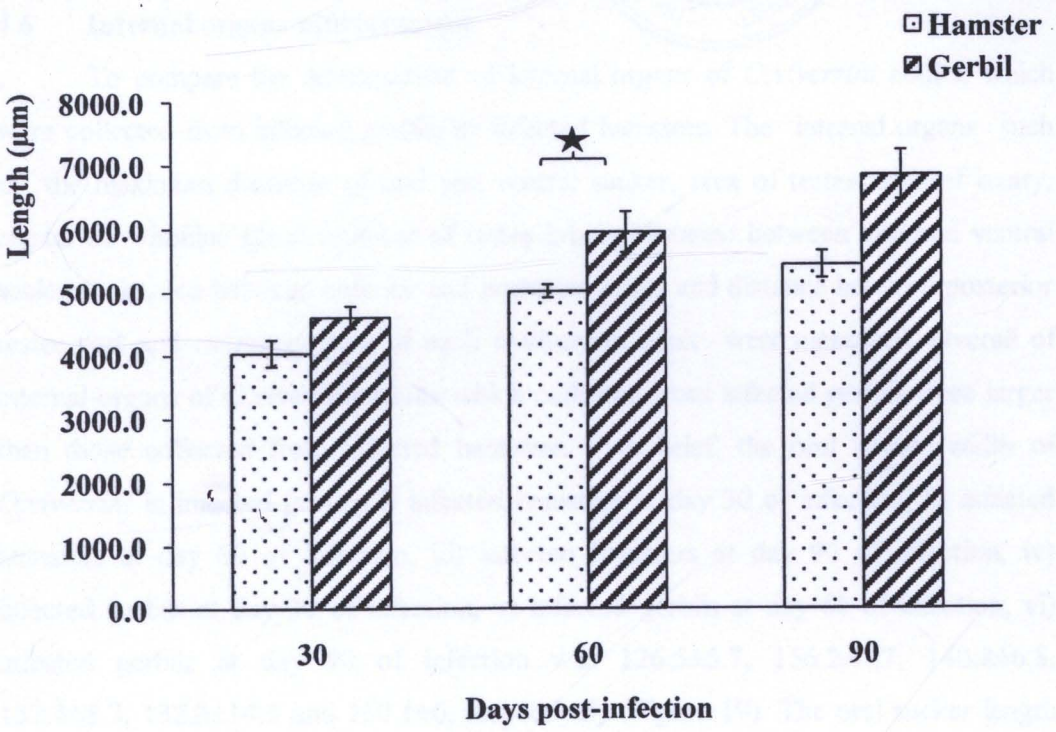

Figure 18 Comparative body length of *O.viverrini* adults which was collected from infected hamsters and gerbils at 30, 60 and 90 days post-infection.

★ Indicates a significant difference within group ($P<0.05$).

4.6 Internal organs measurement

To compare the development of internal organs of *O.viverrini* adults, which were collected from infected gerbils or infected hamsters. The internal organs such as the maximum diameter of oral and ventral sucker, area of testes, area of ovary, cluster of vitelline gland, number of testes lobule, distance between oral and ventral suckers, distance between anterior and posterior testes and distance between posterior testes part and excretory pore of each *O.viverrini* adult were measured. Overall of internal organs of *O.viverrini* adults which collected from infected gerbils were larger than those collected from infected hamsters. In brief, the oral sucker width of *O.viverrini* in infected groups i) infected hamsters at day 30 of infection, ii) infected hamsters at day 60 of infection, iii) infected hamsters at day 90 of infection, iv) infected gerbils at day 30 of infection, v) infected gerbils at day 60 of infection, vi) infected gerbils at day 90 of infection was 126.5±5.7, 156.2±4.7, 140.8±6.8, 152.4±8.7, 132.3±14.5 and 160.1±6, respectively (Figure 19). The oral sucker length of *O.viverrini* in infected groups, i) infected hamsters at day 30 of infection, ii) infected hamsters at day 60 of infection, iii) infected hamsters at day 90 of infection, iv) infected gerbils at day 30 of infection, v) infected gerbils at day 60 of infection, vi) infected gerbils at day 90 of infection was 113.7±4.6, 152±6.3, 113.2±8.7, 133.6±9.9, 60.7±11.6 and 114±12 respectively (Figure 20). The ventral sucker width of *O.viverrini* in infected groups, i) infected hamsters at day 30 of infection, ii) infected hamsters at day 60 of infection, iii) infected hamsters at day 90 of infection, iv) infected gerbils at day 30 of infection, v) infected gerbils at day 60 of infection, vi) infected gerbils at day 90 of infection was 156.9±4.5, 1799.6±7.3, 186.2±12.2, 168.3±8.4, 187.6±13.9 and 196.5±17 respectively (Figure 21). The ventral sucker length of *O.viverrini* in infected groups, i) infected hamsters at day 30 of infection, ii) infected hamsters at day 60 of infection, iii) infected hamsters at day 90 of infection, iv) infected gerbils at day 30 of infection, v) infected gerbils at day 60 of infection, vi) infected gerbils at day 90 of infection was 152.1±4.7, 172.3±5.9, 172.2±10.2, 155.8±7.2, 179.7±13.1 and 182.2±20 respectively (Figure22).

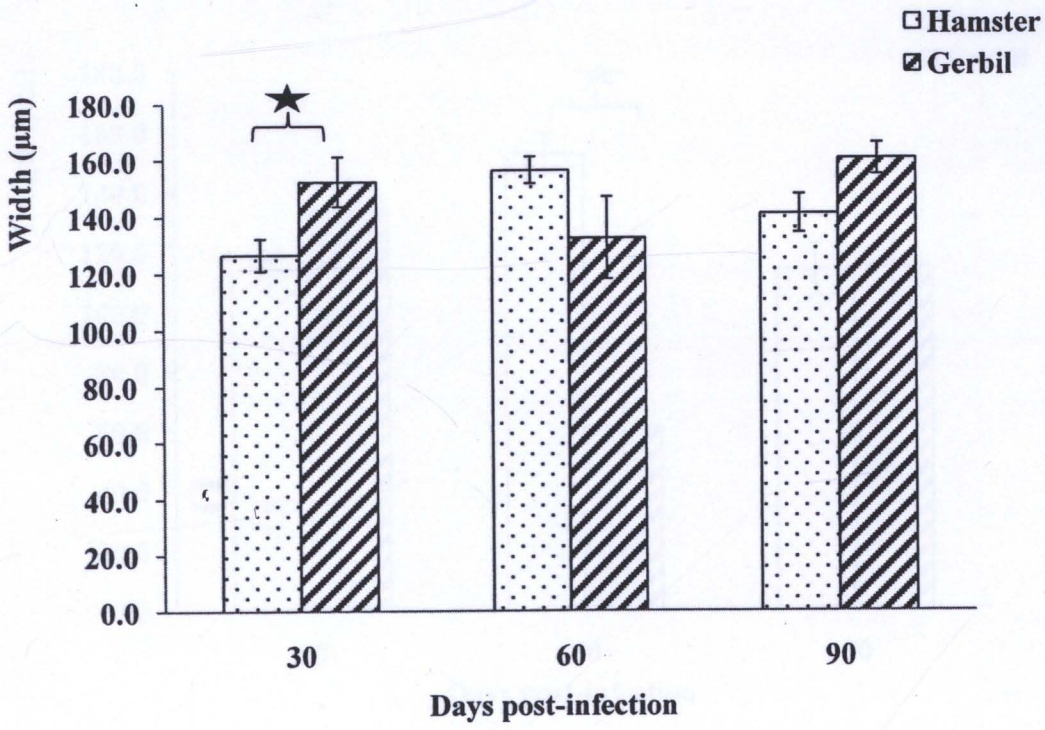

Figure 19 Comparative oral sucker width of *O.viverrini* adults which were collected from infected hamsters and gerbils at 30, 60 and 90 days post-infection.

★ Indicates a significant difference within group ($P<0.05$).

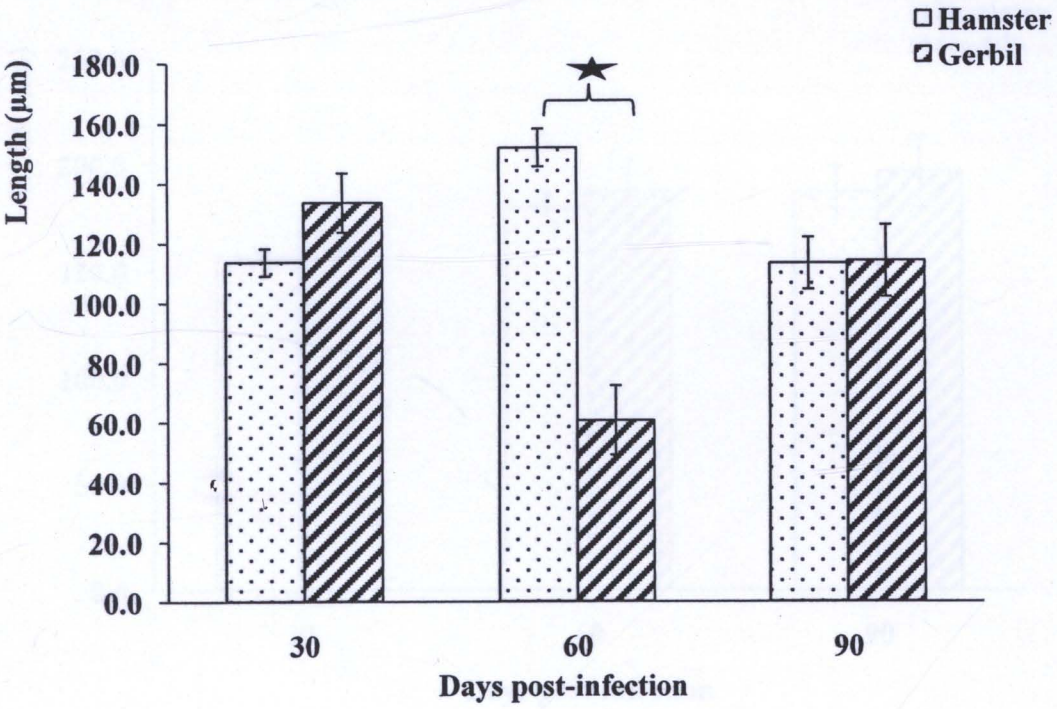

Figure 20 Comparative oral sucker length of *O.viverrini* adults which were collected from infected hamsters and gerbils at 30, 60 and 90 days post-infection.

★ Indicates a significant difference within group ($P<0.05$).

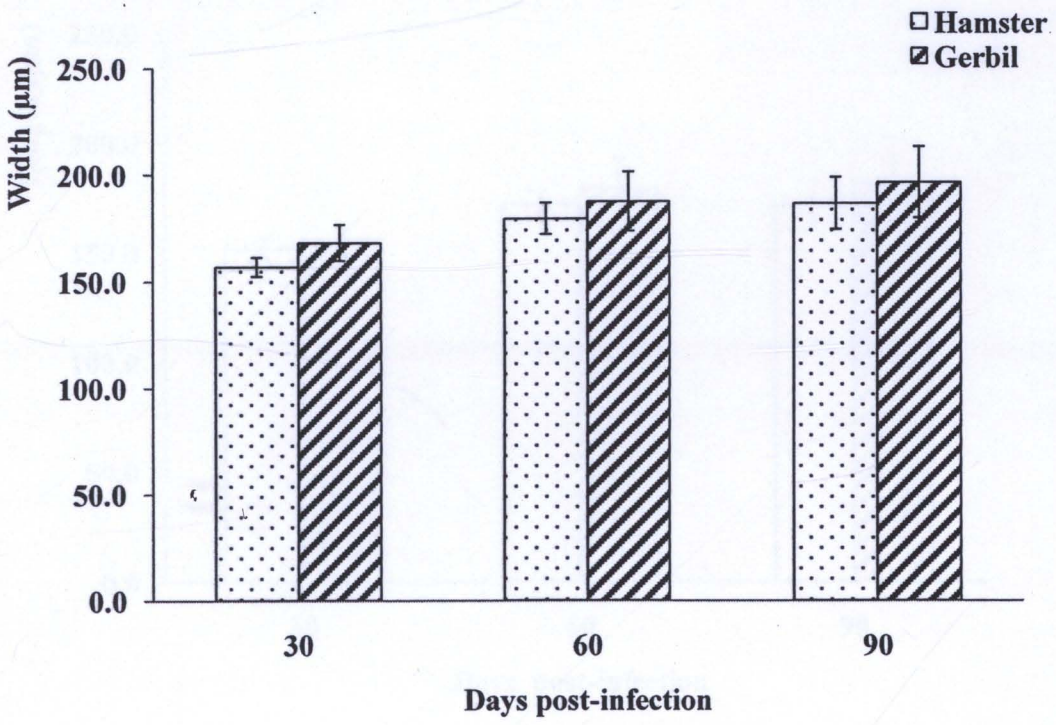

Figure 21 Comparative ventral sucker widths of *O.viverrini* adults which were collected from infected hamsters and gerbils at 30, 60 and 90 days post-infection.

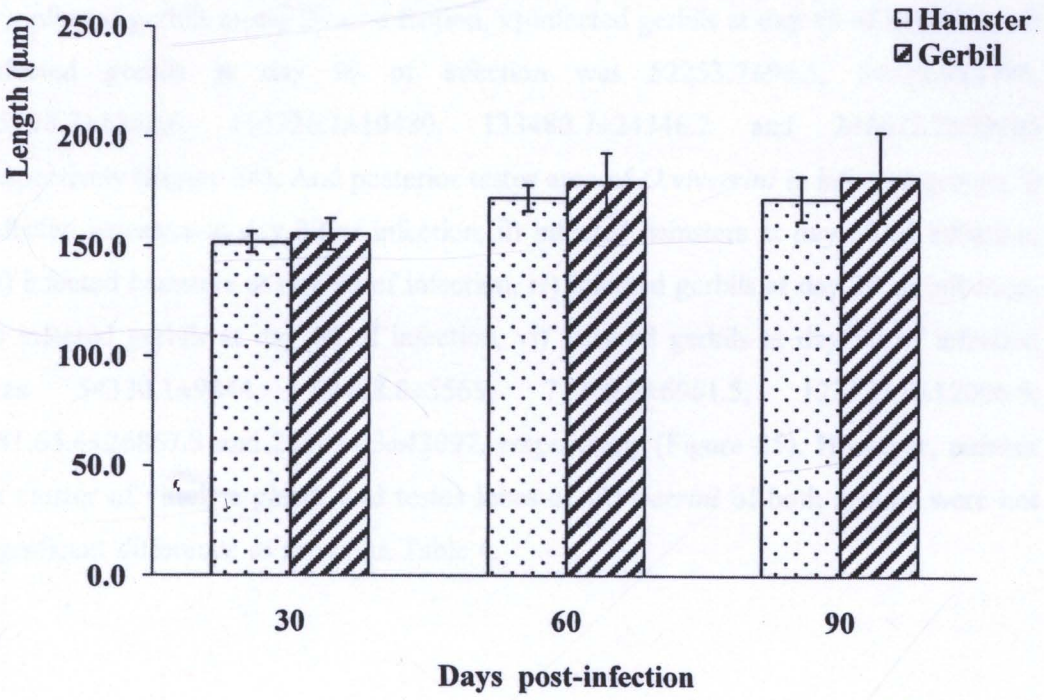

Figure 22 Comparative ventral sucker lengths of *O.viverrini* adults which were collected from infected hamsters and gerbils at 30, 60 and 90 days post-infection.

The area of ovary and both anterior and posterior testes of *O.viverrini* in both infected gerbils and infected hamsters were increasingly with the time manner. Interestingly, the area of ovary of *O.viverrini* adults which were collected from infected gerbils at day 30 was larger 2 fold than those days 90. Moreover, the ovary and both testes areas of parasite from infected gerbils were larger than parasite from infected hamsters at the same time point of observation. The ovary area of *O.viverrini* in infected groups, i) infected hamsters at day 30 of infection, ii) infected hamsters at day 60 of infection, iii) infected hamsters at day 90 of infection, iv) infected gerbils at day 30 of infection, v) infected gerbils at day 60 of infection, vi) infected gerbils at day 90 of infection was 24402±3740, 39326.4±2914, 34122.6±3314, 54915.5±5974, 68388.4±7324.4 and 104585.6±15602 respectively (Figure 23). The anterior testes area of *O.viverrini* in infected groups i) infected hamsters at day 30 of infection, ii) infected hamsters at day 60 of infection, iii) infected hamsters at day 90 of infection,

iv) infected gerbils at day 30 of infection, v) infected gerbils at day 60 of infection, vi) infected gerbils at day 90 of infection was 52253.7±96.5, 54512.9±3796, 65198.7±5862.6, 114726.1±10480, 133480.7±24346.2 and 248622.2±39106 respectively (Figure 24). And posterior testes area of *O.viverrini* in infected groups, i) infected hamsters at day 30 of infection, ii) infected hamsters at day 60 of infection, iii) infected hamsters at day 90 of infection, iv) infected gerbils at day 30 of infection, v) infected gerbils at day 60 of infection, vi) infected gerbils at day 90 of infection was 54330.1±9844, 62408.8±5565, 71209.2±6981.5, 123123.9±12096.9, 141.65.4±26867.3 and 261034.3±43097, respectively (Figure 25). However, number of cluster of vittéline glands and testes lobes of *O.viverrini* of both groups were not significant difference as shown in Table 4.

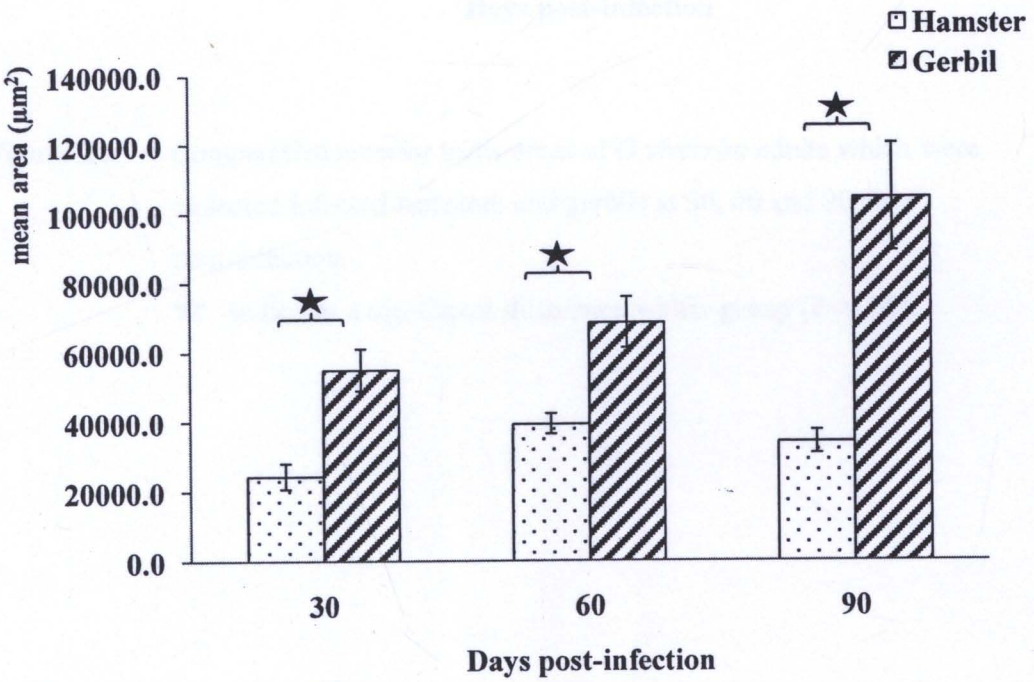

Table 23 Comparative ovary areas of *O. viverrini* adults which were collected from infected hamsters and gerbils at 30, 60 and 90 days post-infection.

★ Indicates a significant difference within group ($P<0.05$).

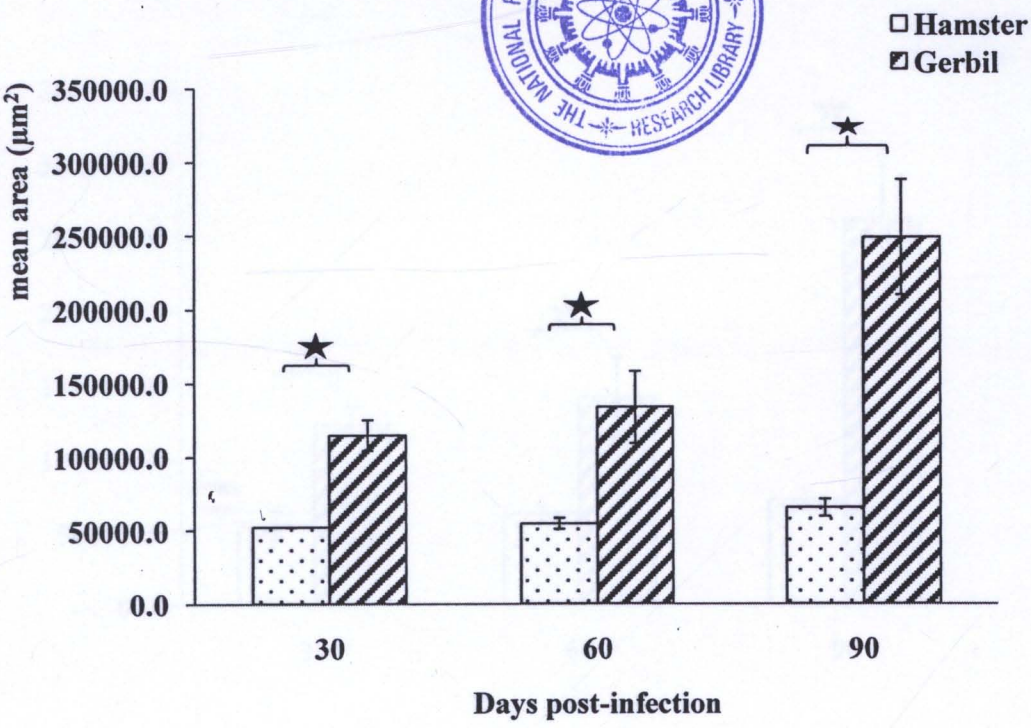

Figure 24 Comparative anterior testis areas of *O.viverrini* adults which were collected infected hamsters and gerbils at 30, 60 and 90 days post-infection.

★ Indicates a significant difference within group ($P<0.05$).

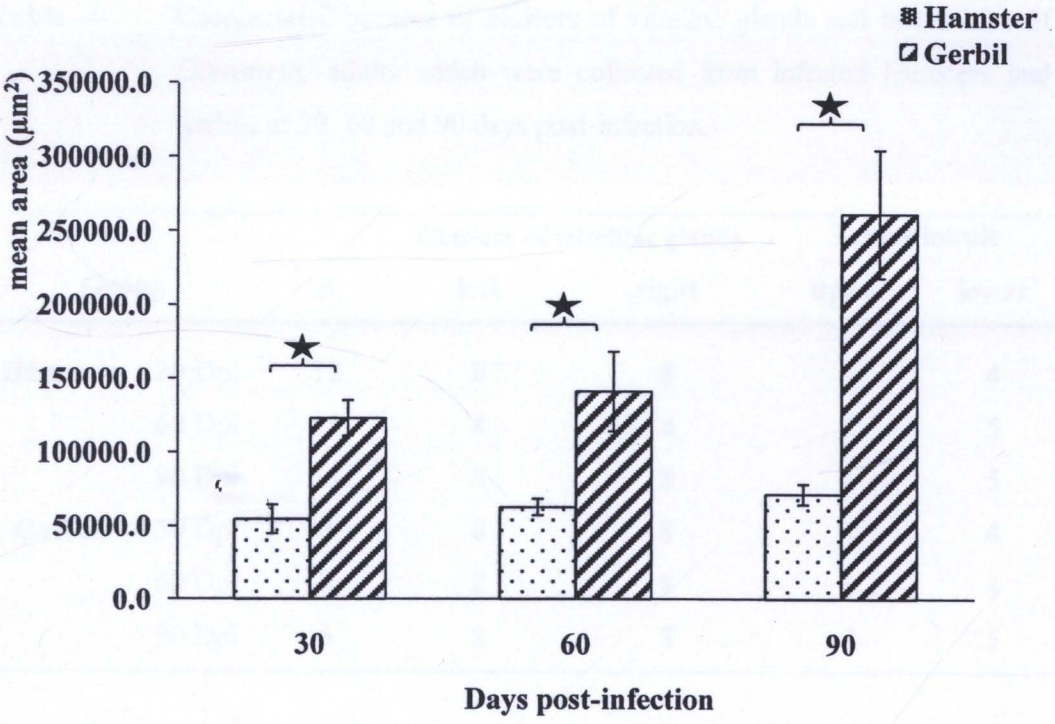

Figure 25 Comparative posterior testis areas of *O.viverrini* adults which were collected from infected hamsters and gerbils at 30, 60 and 90 days post-infection.

★ Indicates a significant difference within group ($P<0.05$).

Table 4 Comparative number of clusters of vitteline glands and testes lobe of *O.viverrini* adults which were collected from infected hamsters and gerbils at 30, 60 and 90 days post-infection.

Group		n	Clusters of vitteline glands		Testes lobule	
			left	right	upper	lower
Hamsters	30 Dpi	12	8	8	4	4
	60 Dpi	15	8	8	4	5
	90 Dpi	13	8	8	4	5
Gerbils	30 Dpi	8	8	8	4	4
	60 Dpi	8	8	8	4	4
	90 Dpi	6	8	8	4	5

The distance between oral and ventral suckers, distance between ovary and testes, distance between anterior testes and posterior testes part and distance between posterior testes part and excretory pore of each *O.viverrini* adult were measured. The distance between oral and ventral suckers of *O.viverrini* adults from the both group were not difference and increased with the time manner. The distance between oral and ventral suckers *O.viverrini* in infected groups, i) infected hamsters at day 30 of infection, ii) infected hamsters at day 60 of infection, iii) infected hamsters at day 90 of infection, iv) infected gerbils at day 30 of infection, v) infected gerbils at day 60 of infection, vi) infected gerbils at day 90 of infection was 957.9±44.4, 1181.1±73.2, 1355.3±46.7 29.8±46.2, 1066.2±141.9 and 1475.9±56 (Figure 26). The distance between ovary and anterior testes of *O.viverrini* was not significant difference at the both group. *O.viverrini* in infected group, i) infected hamsters at day 30 of infection, ii) infected hamsters at day 60 of infection, iii) infected hamsters at day 90 of infection, iv) infected gerbils at day 30 of infection, v) infected gerbils at day 60 of infection, vi) infected gerbils at day 90 of infection was 188.1±29.6, 184.4±23.75, 232.1±28.5, 197±16.9, 223.2±19.9 and 401±17, (Figure 27). The distance between anterior testes and posterior testes part of *O.viverrini* in infected hamster were longer than infected gerbil and increased at day 60 and 90 of observation. The distance

between anterior testes and posterior testes part of *O.viverrini* in infected groups, i) infected hamsters at day 30 of infection, ii) infected hamsters at day 60 of infection, iii) infected hamsters at day 90 of infection, iv) infected gerbils at day 30 of infection, v) infected gerbils at day 60 of infection, vi) infected gerbils at day 90 of infection was 49.4±13.1, 98±13.64, 102.3±15.4, 34.8±10.7, 57±30.7 and 34.9±10 (Figure 28). The distance between posterior testes and excretory pore of each *O.viverrini* at the both group were not difference. *O.viverrini* in infected group, i) infected hamsters at day 30 of infection, ii) infected hamsters at day 60 of infection, iii) infected hamsters at day 90 of infection, iv) infected gerbils at day 30 of infection, v) infected gerbils at day 60 of infection, vi) infected gerbils at day 90 of infection was 274.2±20.1, 403.2±19.98, 439.5±28.3, 297.8±24.3, 392.8±53.1 and 321±30 (Figure 29).

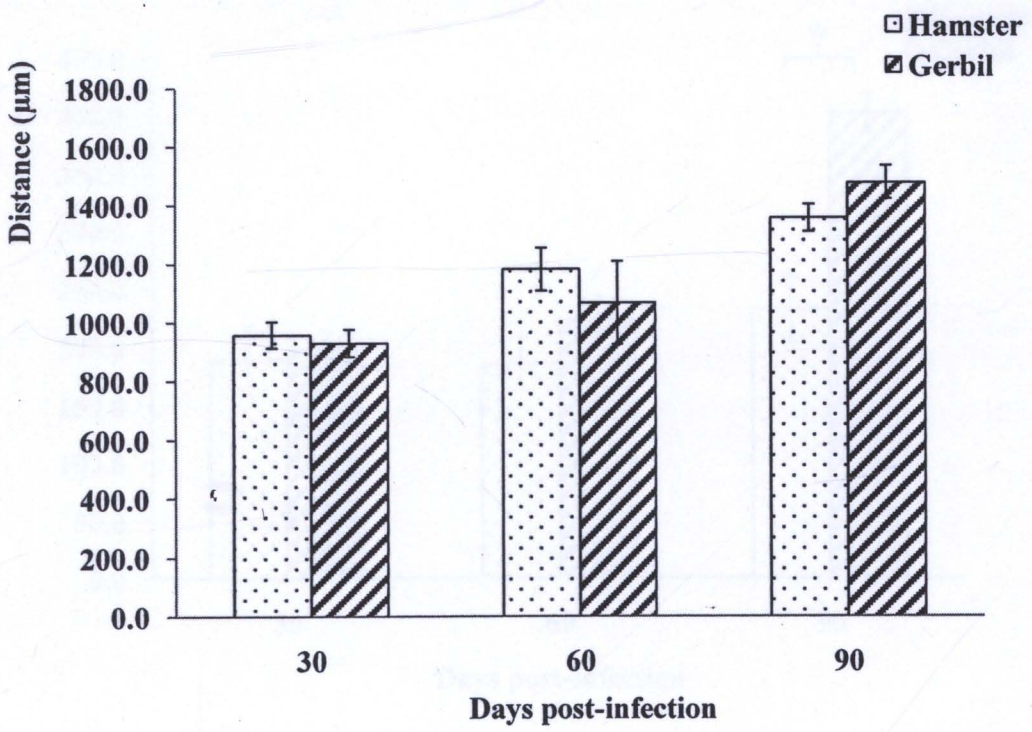

Figure 26 Comparative oral-ventral sucker distance of *O.viverrini* adults which were collected from infected hamsters and gerbils at 30, 60 and 90 days post-infection.

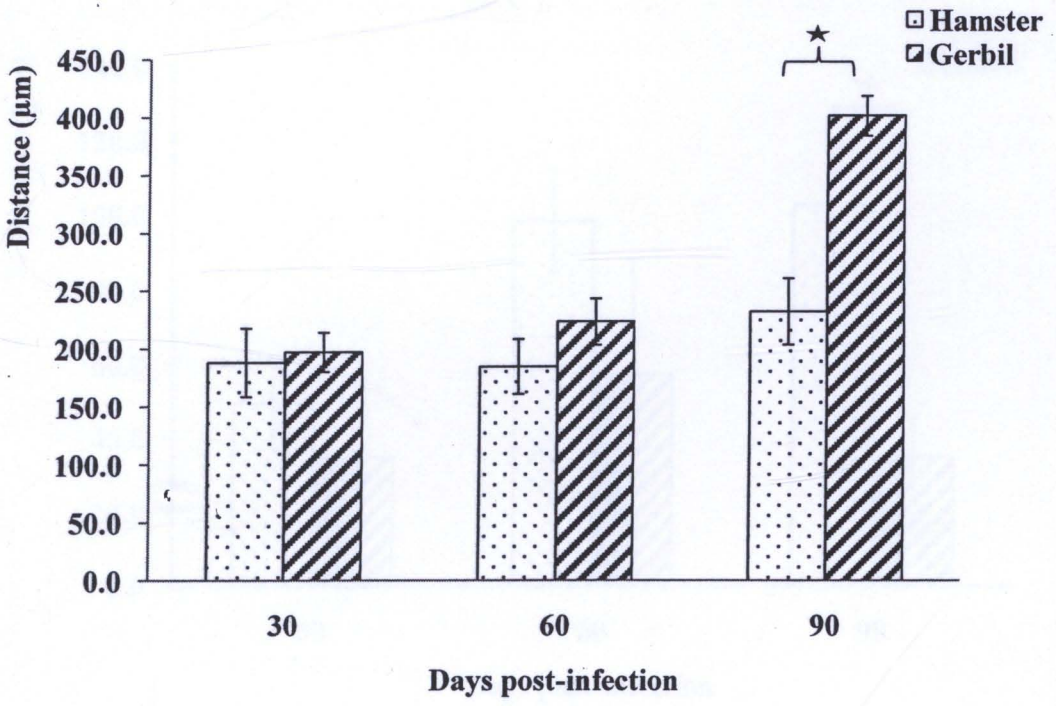

Figure 27 Comparative ovary-anterior testes distance of *O.viverrini* which were collected from infected hamsters and gerbils at 30, 60 and 90 days post-infection.

★ Indicates a significant difference within group ($P<0.05$).

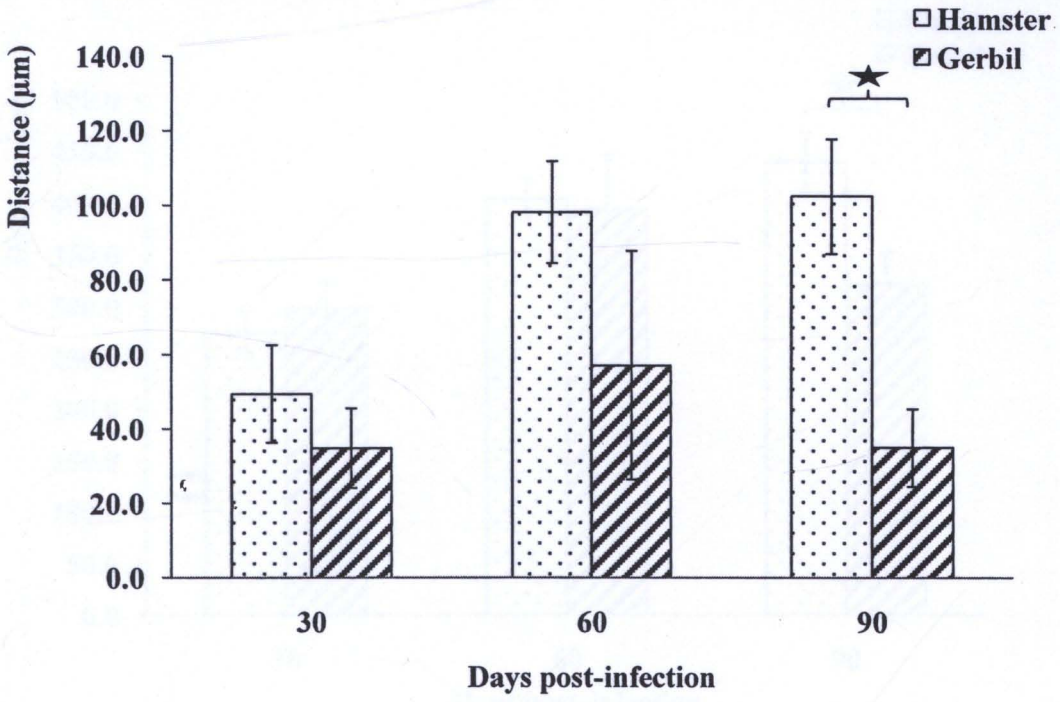

Figure 28 Comparative anterior testes-posterior testes distance of *O.viverrini* adults which were collected from infected hamsters and gerbils at 30, 60 and 90 days post-infection.

★ Indicates a significant difference within group ($P<0.05$).

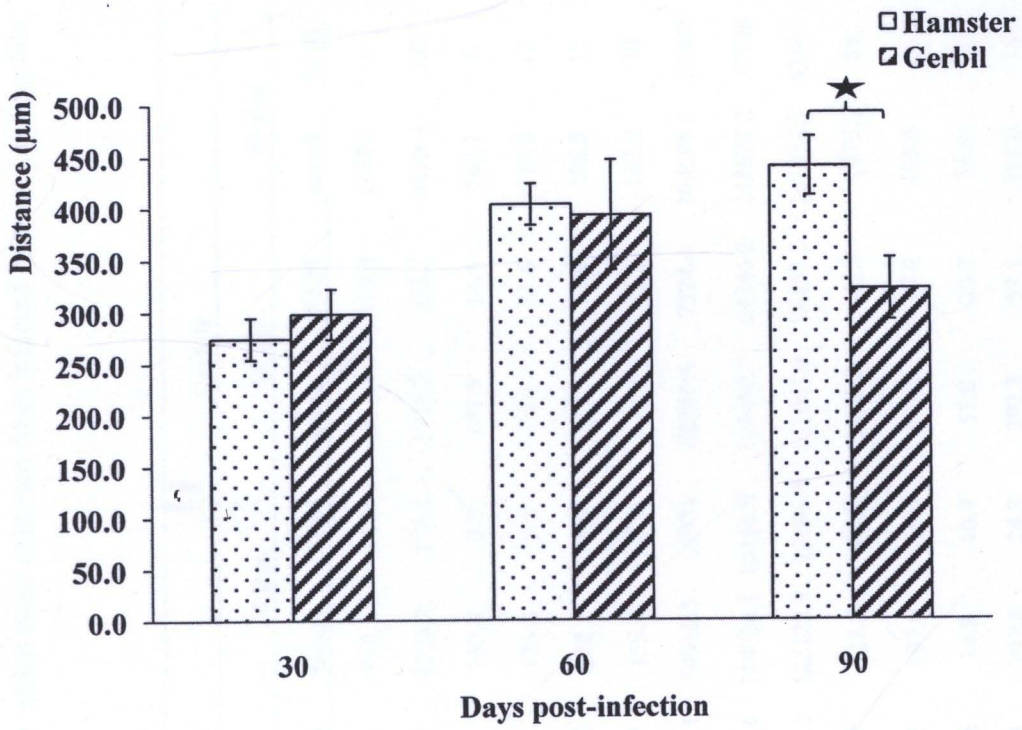

Figure 29 Comparative posterior testes-excretory pore distances of *O.viverrini* adults which were collected from infected hamsters and gerbils at 30, 60 and 90 days post-infection.

★ Indicates a significant difference within group ($P<0.05$).

Table 5 Summary of body sizes and internal organs of *O.viverrini* adults which were collected from infected hamsters and gerbils at 30, 60 and 90 days post-infection.

Characters	Hamsters						Gerbils					
	30 Dpi.		60 Dpi.		90 Dpi.		30 Dpi.		60 Dpi.		90 Dpi.	
	mean	SEM	mean	SEM	mean	SEM	mean	SEM	mean	SEM	mean	SEM
Midbody width	695.9	36.6	1017.5	34.8	1048.2	28.2	918.5	53.2	1335.4	109.8	1240.0	41
Body length	4031.7	209.2	5006.5	103.2	5425.1	215.7	4598.7	175.1	5936.2	323.2	6839.8	388
Oral sucker width	126.5	5.7	156.2	4.7	140.8	6.8	152.4	8.7	132.3	14.5	160.1	6
Oral sucker length	113.7	4.6	152.0	6.3	113.2	8.7	133.6	9.9	60.7	11.6	114.0	12
Ventral sucker width	156.9	4.5	179.6	7.3	186.6	12.2	168.3	8.4	187.6	13.9	196.5	17
Ventral sucker length	152.1	4.7	172.3	5.9	172.2	10.2	155.8	7.2	179.7	13.1	182.2	20
Ovary area	24402.0	3740	39326.4	2914.5	34122.6	3314.1	54915.5	5974	68388.4	7324.4	104595.6	15602
Anterior testes area	52253.7	96.5	54512.9	3796.2	65198.7	5862.6	114726.1	10480.0	133480.7	24346.2	248622.2	39106
Posterior testes area	54330.1	9844.2	62408.8	5564.8	71209.2	6981.5	123123.9	12096.9	141065.4	26867.3	261034.3	43097
Oral-ventral sucker distance	957.9	44.4	1181.1	73.2	1355.3	46.7	929.8	46.2	1066.2	141.9	1475.9	56
Ovary-testes distance	188.1	29.6	184.4	23.7	232.1	28.5	197.0	16.9	223.2	19.9	401.0	17
Testes-testes distance	49.4	13.1	98.0	13.6	102.3	15.4	34.8	10.7	57.0	30.7	34.9	10
Testes-excretory pore distance	274.2	20.1	403.2	20.0	439.5	28.3	297.8	24.3	392.8	53.1	321.0	30

4.7 Thin-layer chromatography

Thin-layer chromatograpy (TLC) was used to determine whether bile components affect the parasite development. Bile fluid of each hamster or gerbil was collected for analysis. Total bile fluids from both groups, hamster and gerbil, were determined using TLC. The spots were observed after heating at 110°C for 5 min. The total bile fluids from both groups were evaluated base on standard spots (CA and CDCA). The plate was examined by spraying with 2% w/v vanilline and 10% v/v sulphuric acids which showed distinct separated three spots; ß-sitosterol (lane 1), CA (lane 4) and CDCA (lane 5) as standard control respectively. Bile fluid of hamster (lane 2) showed separated 4 spots, their R_f values and colour equivalent to ß-sitosterol, CDCA, CA and an unknown. For gerbil (lane 3), separated 2 spots were detected, one of which the R_f values and color equivalent to ß-sitosterol and an unknown (Table 6 and Figure 30).

The TLC system clearly differentiated the bile fluids of hamster and gerbil. From the hamster's, 4 components were clearly separated which one spots at the R_f values of 0.85, 0.60, 0.52 and 0.15. At R_f values 0.52 and 0.58, they were CA and CDCA, while the R_f values 0.85 is equivalent to ß-sitosterol which was used to a marker. For the gerbil bile fluid CA and CDCA could not be detected, whereas those more polar components were found much more than that of hamsters.

Table 6 Thin-layer chromatograpy was examined by spraying the plate with 2% w/v vanilline and 10% v/v sulphuric acids.

Characteristic	Lane	Spot component	color	R_f	compound
Stardard markers					
ß-sitosterol	1	1	Purple	0.85	Known
CA	4	1	Purple	0.52	Known
CDCA	5	1	Blue	0.58	Known
Sample					
Bile fluid of hamsters	2	4	Purple	0.85	Known
			Blue	0.60	Known
			Purple	0.52	Known
			Purple	0.15	Unknown
Bile fluid of gerbils	3	2	Purple	0.85	Known
			Purple	0.15	Unknown

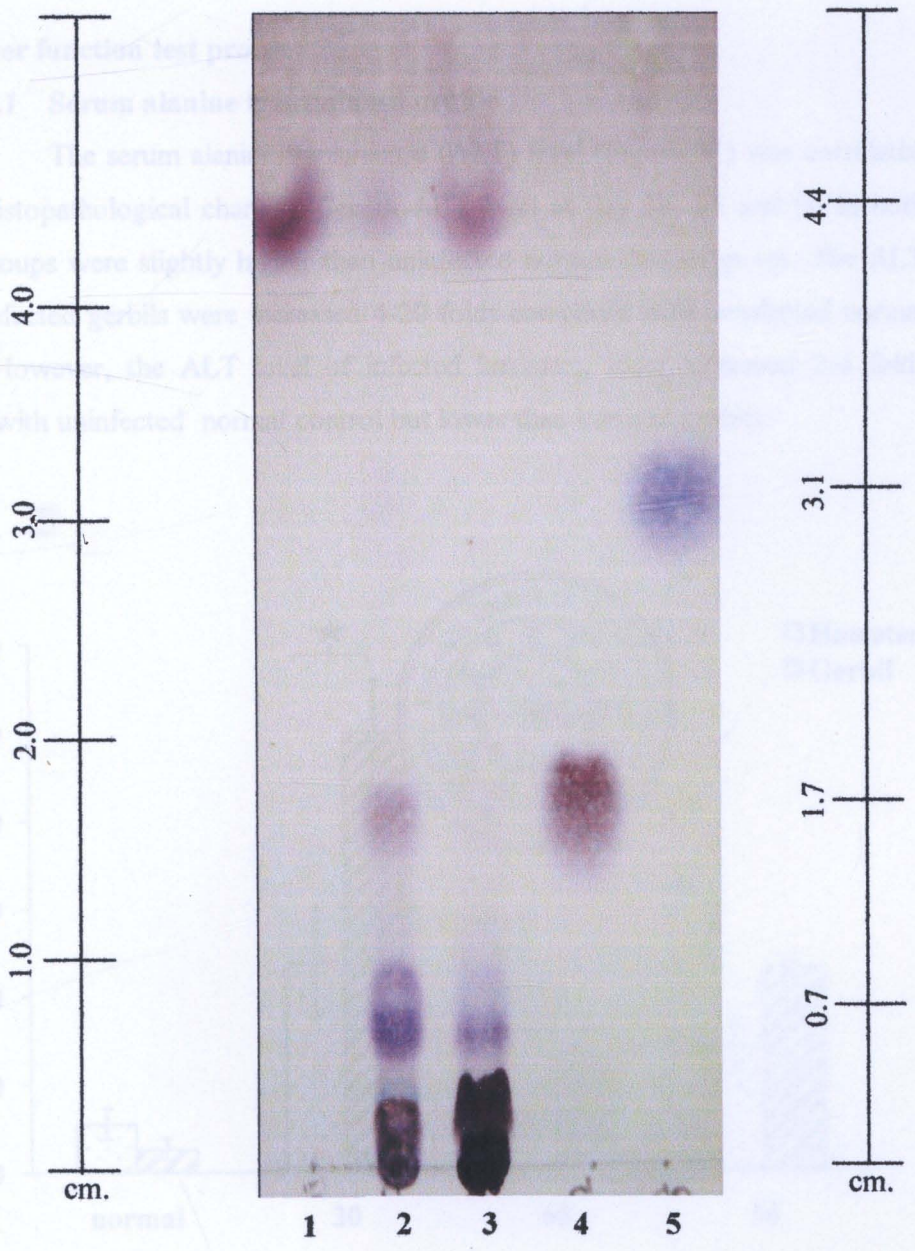

Figure 30 Thin-layer chromatogram of total hamster bile fluid (lane 2) and total gerbil bile fluid (lane3). Standards (lane 1; 4-5): ß-sitosterol, CA and CDCA respectively. Stationary phase: siliga gel aluminium plate. Mobile phase: Hexane: Ethyl acetate: Acetic acid: Methanol (7:23:3:2). Detector: 2% w/v vanilline and 10% v/v sulphuric acids heated at 110°C for 5 min.

4.8 Liver function test profile

4.8.1 Serum alanine transminase profile

The serum alanine transminase (ALT) level (Figure 31) was correlated with the histopathological changes. Serum ALT level at day 30, 60 and 90 in both infected groups were slightly higher than uninfected normal control group. The ALT levels of infected gerbils were increased 4-20 folds compared with uninfected normal control. However, the ALT level of infected hamsters, were increased 2-4 folds compared with uninfected normal control but lower than infected gerbils.

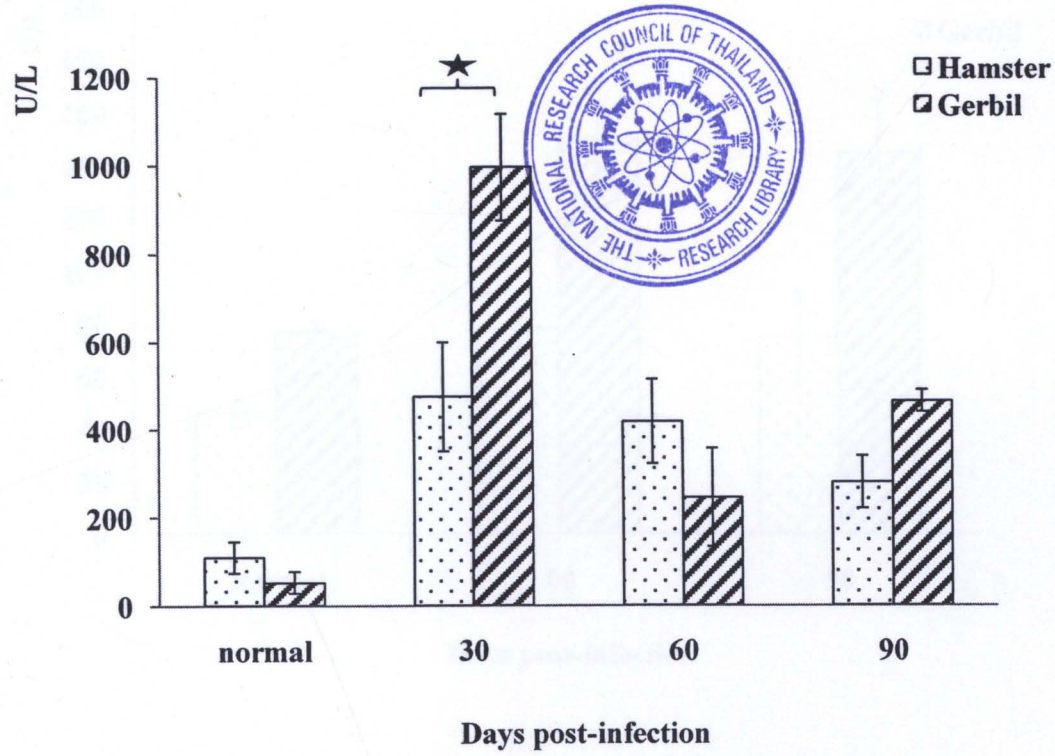

Figure 31 Serum alanine transminase level in uninfected normal control and infected hamsters and gerbils at 30, 60 and 90 days post-infection.

★ Indicates a significant difference within group ($P<0.05$).

4.8.2 Serum alkaline phosphatase profile

The serum alkaline phosphatase (ALP) level (Figure 32) was correlated with the histopathological changes. Serum ALP level at day 60 and 90 in both infected hamster and gerbils were slightly higher than uninfected normal control. In infected gerbils, the ALP levels were increased by 1-2 folds compared with uninfected normal control. However, the ALP level in infected hamsters, were slightly increased compared with uninfected normal control but seem to lower than infected gerbils.

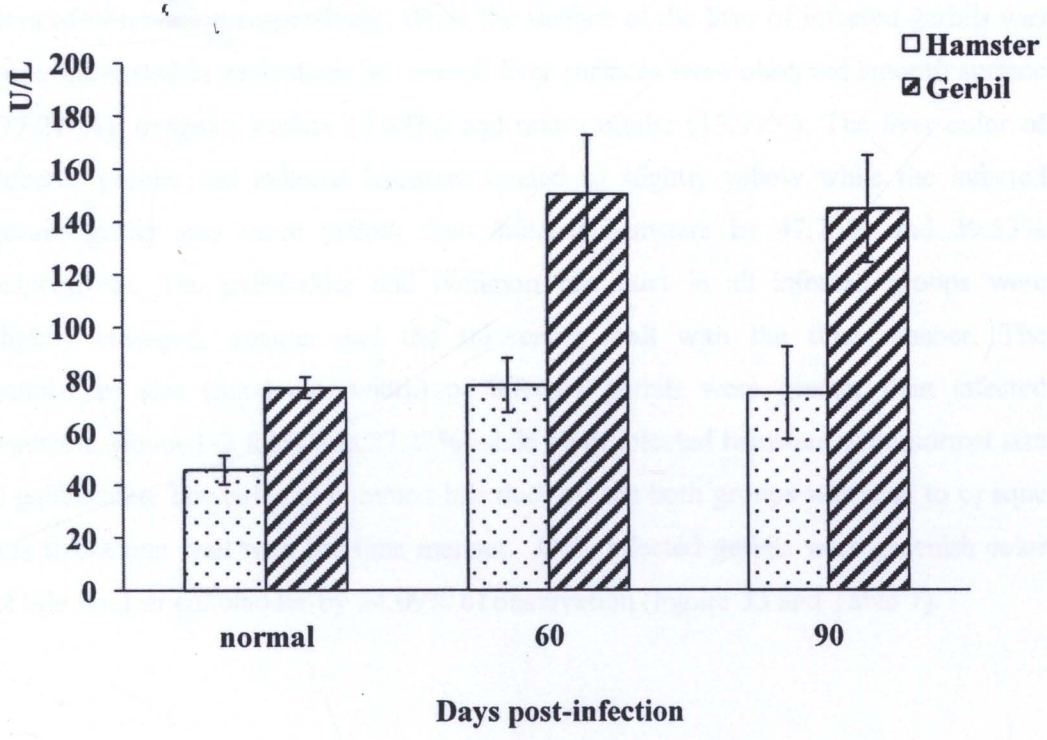

Figure 32 Serum alkaline phosphatase level in uninfected normal control and infected hamsters and gerbils at 60 and 90 days post-infection.

4.9 Gross pathological changes of the hepatobiliary system

The liver from infected hamsters and gerbils were harvested and monitored at day 30, 60 and 90 post-infection. The gross appearance such as liver surface, liver color, gallbladder size (maximum width), common bile duct (color) and color of bile fluid in gallbladder were observed. The gross appearance in the group of infected hamsters was similar in liver surface, liver color, gallbladder size and the color of common bile duct and bile acids in gallbladder to the uninfected normal control (Figure 33). The surface of all groups seemed to smooth but if comparative surface of all stages together, the liver tended to irregular by 33.33% and 15.38% at 60 and 90 days of observation respectively. While the surface of the liver of infected gerbils was quite fluctuated in each stage but overall liver surfaces were observed smooth surface (77.27 %), irregular surface (6.82%) and micro odular (15.91%). The liver color of infected gerbils and infected hamsters tended to slightly yellow while the infected gerbils group was more yellow than infected hamsters by 47.73% and 39.53% respectively. The gallbladder and common bile duct in all infected groups were slightly enlarged, opaque and the thickening wall with the time manner. The gallbladder size (maximum width) of infected gerbils were greater than infected hamsters (about 1-2 fold) was 27.27% while all of infected hamsters were normal size if gallbladder. The color of common bile duct of the both groups was tend to opaque and thickening wall with the time manner. Only infected gerbils were greenish color of bile fluid in gallbladder by 34.09% of observation (Figure 33 and Table 7).

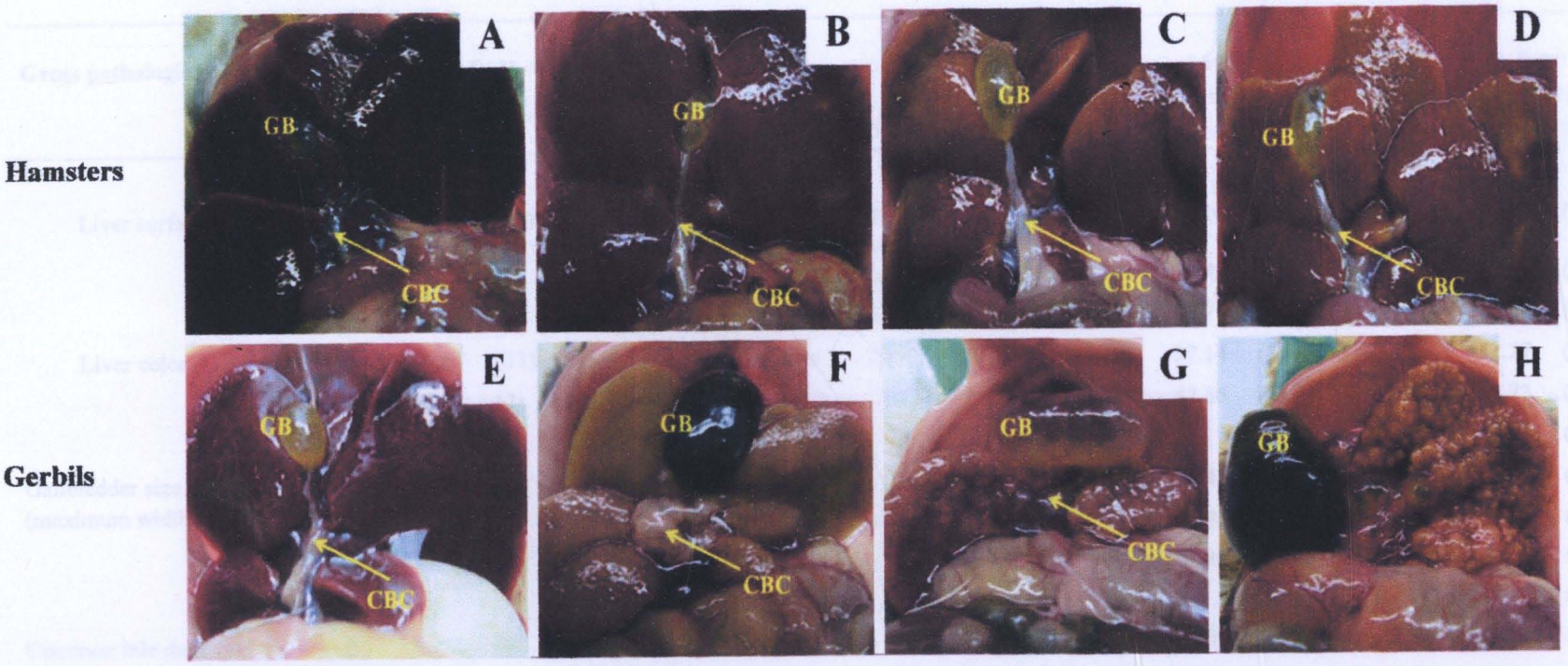

Figure 33 Gross appearance of the color and surface liver, gallbladder (GB) and common bile duct (CBC) in the group of hamsters and gerbils infected with *O.viverrini* at day 30 (B; F), 60 (C; G) and 90 (D; H) post-infection compared with uninfected normal control (A; E).

Table 7 Gross pathological feature of gallbladder and liver criteria by macroscopic observation.

Gross pathological	score	Hamsters								Gerbils					
		30 Dpi (n=15)		60 Dpi (n=15)		90 Dpi (n=13)		Total (n=43)		30 Dpi (n=15)		60 Dpi (n=14)		90Dpi (n=15)		Total (n=44)	
		%	(n)	%	(n)	%	(n)	%	(n)	%	(n)	%	(n)	%	(n)	%	(n)
Liver																	
Liver surface	1	100.0	(15)	66.67	(10)	84.62	(11)	83.72	(36)	80	(12)	57.14	(8)	93.3	(14)	77.27	(34)
	2			33.33	(5)	15.38	(2)	16.28	(7)	13.33	(2)	7.14	(1)	0.00	(0)	6.82	(3)
	3							0.00	(0)	6.67	(1)	35.7	(5)	6.67	(1)	15.91	(7)
Liver color	0	86.67	(13)	46.67	(7)	46.15	(6)	60.47	(26)	46.67	(7)	57.14	(8)	66.67	(10)	52.27	(23)
	1	13.33	(2)	53.33	(8)	53.85	(7)	39.53	(17)	53.33	(8)	42.86	(6)	33.33	(5)	47.73	(21)
	2							0.00	(0)							0.00	(0)
Gallbladder size (mm.) (maximum width)	0	100.0	(15)	100	(15)	100	(13)	100	(43)	26.67	(4)	71.43	(10)	66.67	(10)	54.55	(24)
								0	(0)	33.33	(5)	7.14	(1)	13.33	(2)	18.18	(8)
								0	(0)	40.00	(6)	21.43	(3)	13.33	(2)	25.00	(11)
								0	(0)					6.67	(1)	2.27	(1)
Common bile duct (color)	0	53.33	(8)	13.33	(2)	46.15	(6)	37.21	(16)	6.67	(1)	14.29	(2)	6.67	(1)	9.09	(4)
	1	46.67	(7)	53.33	(8)	30.77	(4)	44.19	(19)	33.33	(5)	42.86	(6)	53.33	(8)	43.18	(19)
	2			33.33	(5)	23.08	(3)	18.60	(8)	60.00	(9)	42.86	(6)	40.00	(6)	47.73	(21)
Bile acids color in gallbladder	1	100.0	(15)	66.67	(10)	76.92	(10)	81.40	(35)	26.67	(4)	35.71	(5)	33.33	(5)	31.82	(14)
	2			33.33	(5)	23.08	(3)	18.60	(8)	33.33	(5) (6)	35.71	(5)	33.33	(5)	34.09	(15)
	3							0.00	(0)	40.00		28.57	(4)	33.33	(5)	34.09	(15)

4.10 The histopathological changes of the hepatobiliary system

According to the normal control histopathological changes of gallbladder, liver and pancreas of the hamsters and gerbils's hepatobiliary system was monitored at 30, 60 and 90 days. The histopathological changes correlated with the gross appearance results as followed the gallbladder of infected gerbils were observed scattering mononuclear cells, lymphocytes and eosinophils with or without plasma cells at day 30 and 60 (100% , 60 % respectively). The minimal of PMN's cells (eosinophil) and mononuclear cell (lymphocyte) at day 60 and 90 were 40% and 100% respectively (Figure 34 and Table 26). The globlet cells metaplasia were observed in both infected hamsters and gerbils by 64.3% and 91.6% respectively (Table 26). The gallbladder fibrosis in infected gerbils was 41.7% while gallbladder epithelial dysplasia and fibrosis of infected hamster could not be observed (Figure 34 and Table 8).

The liver pathological changes of infected hamsters were similar to our previous report (Boonmars et al, 2010). In brief, the portal triad and hepatic tissue of infected hamsters tended to increasing of inflammatory cells 2/3 of portal triad more than infected gerbils at the same time point (Figure 36 and Table 9). In addition, hepatic bile ducts size of infected hamsters were moderated dilatation or located with a parasite (88.2%) while, the intrahepatic bile ducts size of all infected gerbils were minimal dilatation (52.2%) (Table 9). For hepatic bile duct proliferations were observed in both infected hamsters and gerbils. In infected gerbils showed scattering about 34.8% while, those of observed in infected hamsters was about 50% (Table 9). Moreover, infected hamsters were observed focal inflammation more than infected gerbils (Table 9). Fibrous bridge at portal area with incomplete nodule or cirrhosis was observed in some infected gerbils but not infected hamsters at the same time points of observation (Figure 35 and Table 9). The infected hamsters, parasites were observed within the gallbladder, large hepatic bile duct, common bile duct, intrahepatic bile ducts and pancreatic duct at all time points of observation (Figure 37). The parasites in infected gerbils were located at the same areas of infected hamsters excepted intrahepatic bile ducts.

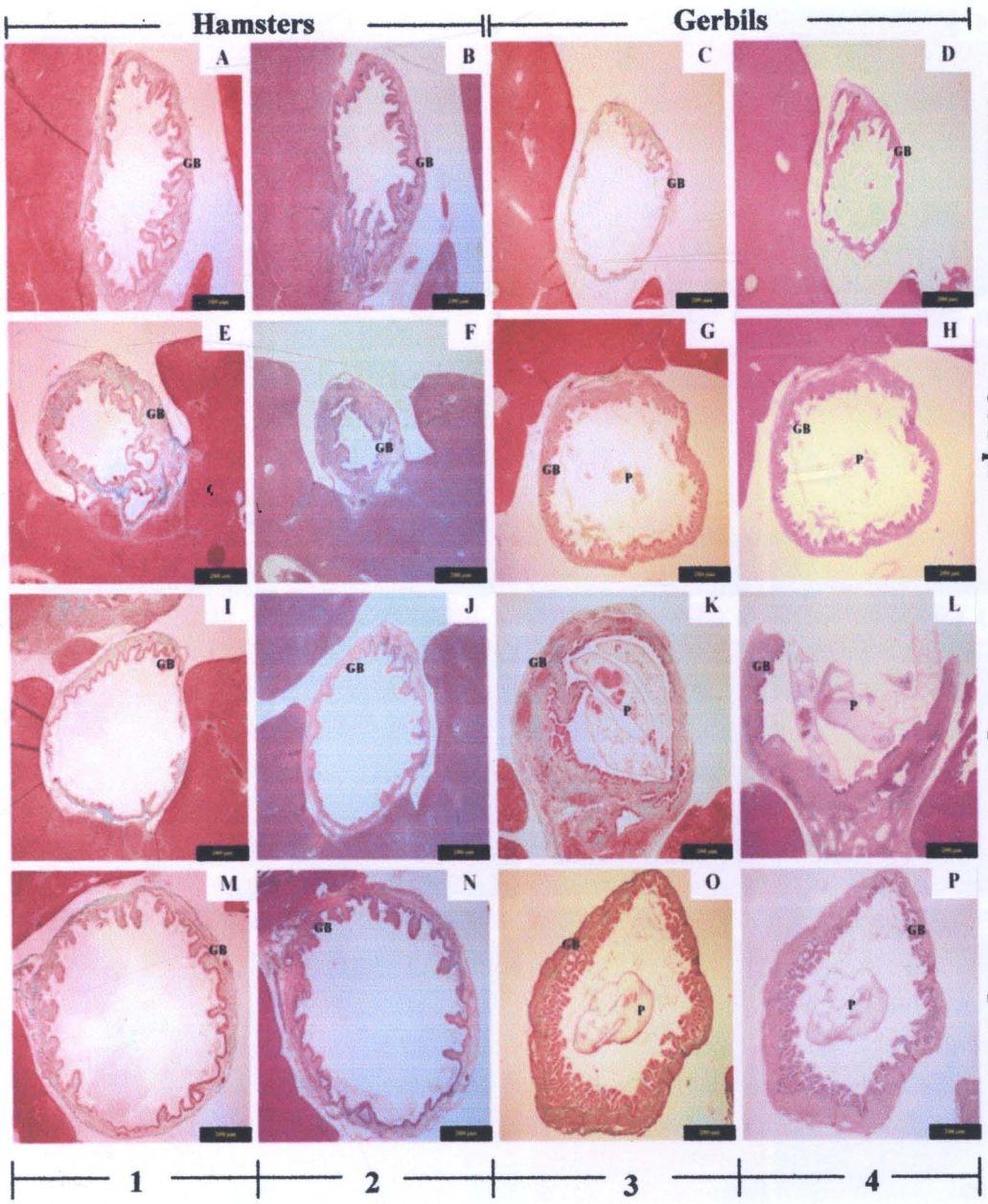

Figure 34 Histopathological changes of gallbladder in uninfected normal control hamsters (A; B) and gerbils (C; D) compared with infected hamsters (E;F;I;J;M;N) and gerbils (G;H;K;L;O;P). Lane 1, 3: Gomori's trichrome stain; Lane 2, 4: H&E stain. GB: gallbladder; P: parasite.

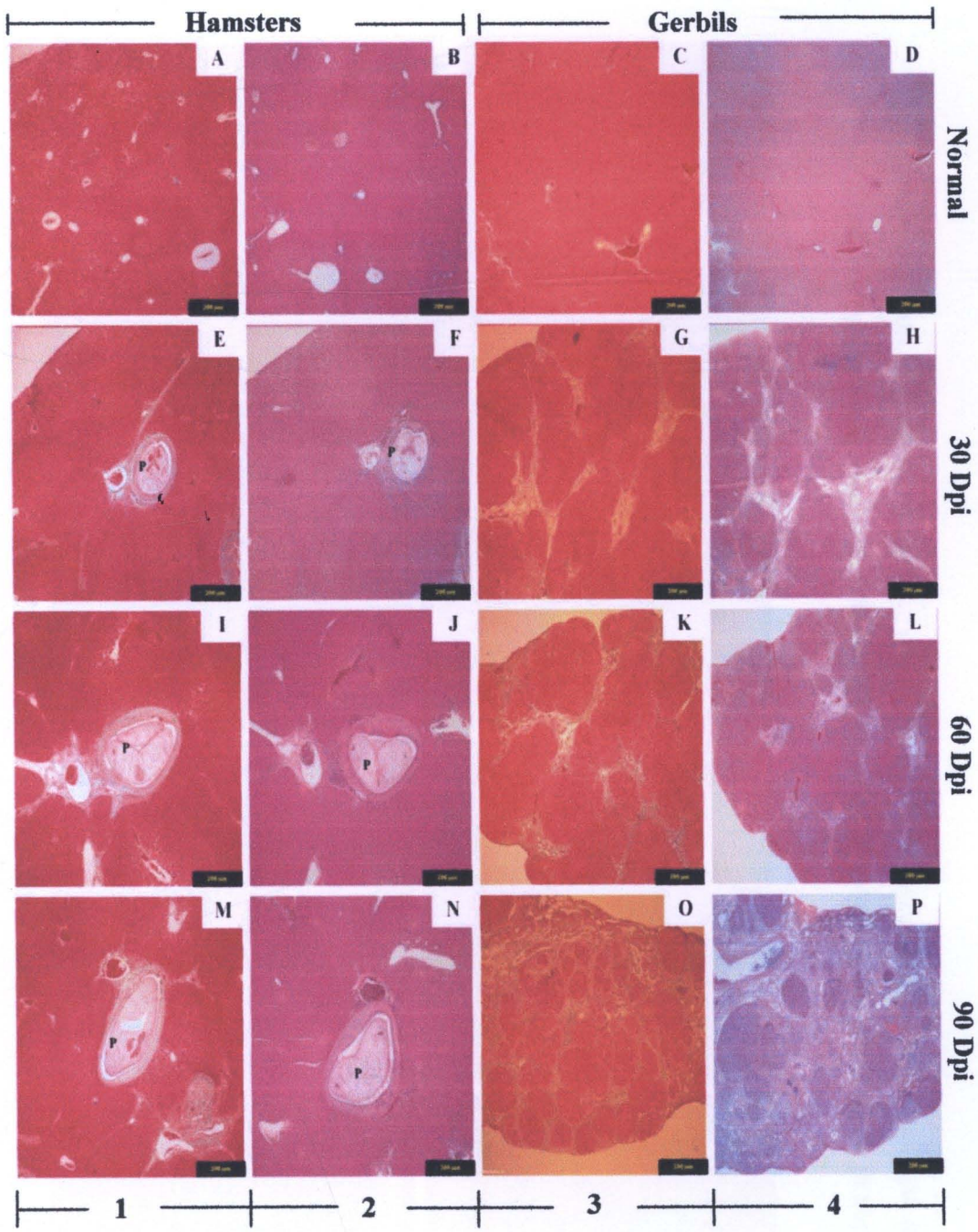

Figure 35 Histopathological changes of liver. Uninfected normal control hamsters (A; B) and gerbils (C; D) compared with the infected hamsters and gerbils at day 30 (E; F; G; H), 60 (I; J; K; L) and 90 (M; N; O; P). Lane 1, 3: Gomori's trichrome stain; Lane2, 4: H&E stain; P: parasite.

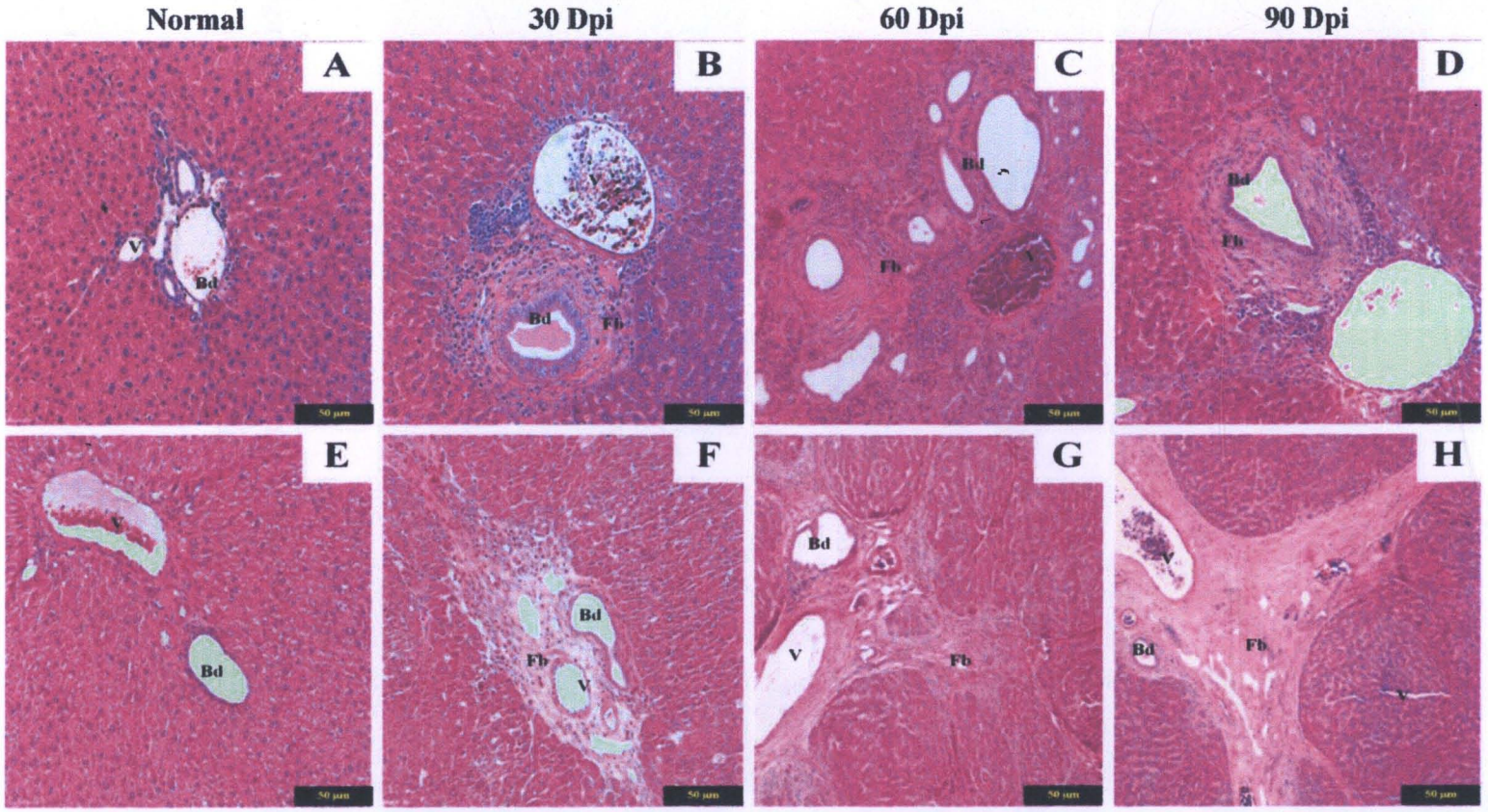

Figure 36 High magnification of histopathological changes of liver. Uninfected normal control hamsters (A) and gerbils (E) comparedwith the infected hamsters and gerbils at day 30 (B; F), 60 (C; G) 90 (D; H). Bd: bile duct; Fb: fibrosis; V: vessel.

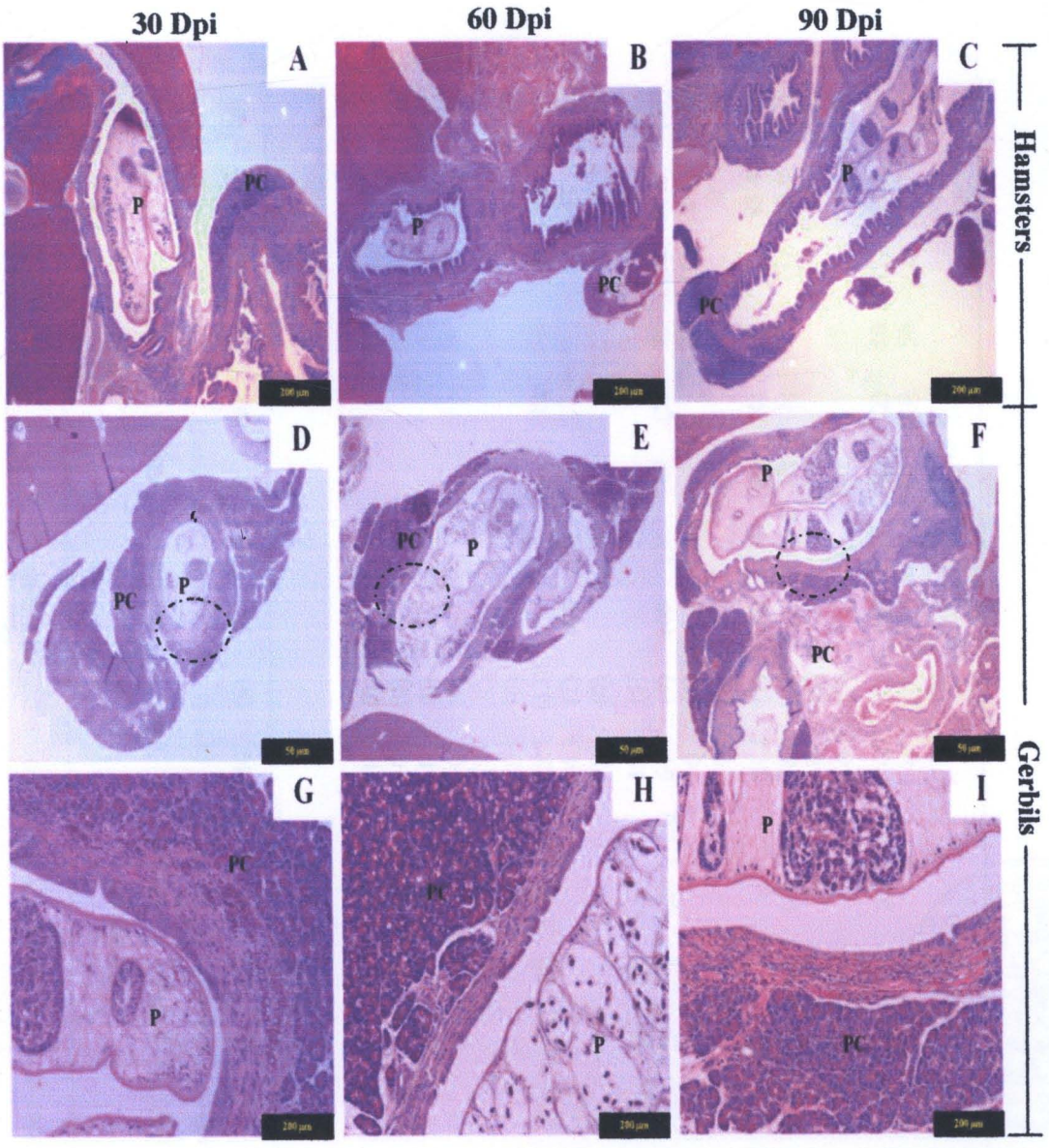

Figure 37 Histopathological changes of pancreas in infected hamsters (A-C) and gerbils (D-I) at 30, 60 and 90 days post-infection. P: parasite; PC: pancreas.

Table 8 Histological features of gallbladder grading criteria by microscopic observation.

Histopathological	score	Hamsters								Gerbils							
		30 Dpi (n=5)		60 Dpi (n=4)		90Dpi (n=5)		Total (n=14)		30 Dpi (n=3)		60 Dpi (n=5)		90 Dpi (n=4)		Total (n=12)	
		%	(n)	%	(n)	%	(n)	%	(n)	%	(n)	%	(n)	%	(n)	%	(n)
Gallbladder																	
	Acute																
Inflammation	0							0	(0)							0	(0)
	1							0	(0)							0	(0)
	2							0	(0)							0	(0)
	3							0	(0)							0	(0)
	Chronic																
	0							0	(0)							0	(0)
	1	60	(3)	100	(4)	100	(5)	85.7	(12)			40	(2)	100	(4)	50	(6)
	2	40	(2)					14.3	(2)	100	(3)	60	(3)			50	(6)
	3							0	(0)							0	(0)
Globlet cell metaplasia	0	60	(3)	50	(2)			35.7	(5)							0	(0)
	1	40	(2)	50	(2)	100	(5)	64.3	(9)	100	(3)	100	(5)	75	(3)	91.6	(11)
	2							0	(0)					25	(1)	8.4	(1)
	3							0	(0)							0	(0)
Epithelial dysplasia	0	60	(3)					21.4	(3)	100	(3)	80	(4)	75	(3)	83.4	(10)
	1	40	(2)	100	(4)	100	(5)	78.6	(11)			20	(1)	25	(1)	16.6	(2)
	2							0	(0)							0	(0)
	3							0	(0)							0	(0)
Fibrosis	0	100	(5)	100	(4)	100	(5)	100	(14)	100	(3)	40	(2)	50	(2)	58.3	(7)
	1							0	(0)			60	(3)	50	(2)	41.7	(5)
	2							0	(0)							0	(0)
	3							0	(0)							0	(0)

Table 9 Histological feature of liver biopsy and grading criteria by microscopic observation.

Histopathological	score	Hamsters								Gerbils							
		30 Dpi (n=14)		60 Dpi (n=9)		90 Dpi (n=11)		Total (n=34)		30 Dpi (n=7)		60 Dpi (n=9)		90Dpi (n=7)		Total (n=23)	
		%	(n)	%	(n)	%	(n)	%	(n)	%	(n)	%	(n)	%	(n)	%	(n)
Portal triad and hepatic tissue	0							0	(0)	14.3						0	(0)
Portal inflammation	1			66.7	(6)	27.3	(2)	23.5	(8)	85.7	(1)	77.8	(7)	85.7	(6)	60.9	(14)
	2	57.1	(8)	33.3	(3)	72.7	(8)	55.9	(19)		(6)	22.2	(2)	14.3	(1)	39.1	(9)
	3	42.9	(6)					17.6	(6)							0	(0)
Fibrosis	0																
	1	100	(14)	100	(9)	100	(11)	100	(34)					28.6	(2)	8.7	(2)
	2									14.3	(1)	11.1	(1)	28.6	(2)	17.4	(4)
	3									14.3	(1)					4.3	(1)
	4									57.1	(4)					17.4	(4)
	5											66.7	(6)	28.6	(2)	34.8	(8)
	6									14.3	(1)	22.2	(2)	14.3	(1)	17.3	(4)
Hepatic bile duct size	0									28.6	(2)	11.1	(1)			13.0	(3)
	1									57.1	(4)	55.6	(5)	42.9	(3)	52.2	(12)
	2	85.7	(12)	77.8	(7)	100	(11)	88.2	(30)	14.3	(1)	33.3	(3)	57.1	(4)	34.8	(8)
	3	14.3	(2)	22.2	(2)			11.3	(4)							0	(0)

Table 9 Histological feature of liver biopsy and grading criteria by microscopic observation (Cont.).

Histopathological	score	Hamsters								Gerbils							
		30 Dpi (n=14)		60 Dpi (n=9)		90 Dpi (n=11)		Total (n=34)		30 Dpi (n=7)		60 Dpi (n=9)		90Dpi (n=7)		Total (n=23)	
		%	(n)	%	(n)	%	(n)	%	(n)	%	(n)	%	(n)	%	(n)	%	(n)
Portal triad and hepatic tissue																	
Hepatic bile duct proliferation	0							0	(0)	28.6	(2)	11.1	(1)	42.9	(3)	13.0	(3)
	1	78.6	(11)	22.2	(2)	36.4	(4)	50	(17)	57.1	(4)	55.6	(5)	57.1	(4)	52.2	(12)
	2	21.4	(3)	77.8	(7)	63.6	(7)	50	(17)	14.3	(1)	33.3	(3)			34.8	(8)
	3							0	(0)							0	(0)
Focal inflammation	0							0	(0)							0	(0)
	1							0	(0)			55.6	(5)	85.7	(6)	47.8	(11)
	2	92.9	13	100	9	100	11	97.1	(33)	85.7	(6)	44.4	(4)	14.3	(1)	47.8	(11)
	3	7.1	1				0	2.9	(1)	14.3	(1)					4.4	(1)
	4							0	(0)							0	(0)