UNIVERSITI PUTRA MALAYSIA

A STUDY ON THE PATHOLOGY OF KIDNEYS OF SWINE SLAUGHTERED AT SHAH ALAM ABATTOIR

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BY

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ABSTRACT

A survey on the pathology of kidneys of swine condemned at slaughter at Shah Alam abattoir was done over 2-week period. A total of 342 kidneys of 252 pigs were collected and were identified whether the involvement was unilateral or bilateral. Pathological, microbiological and cultural examinations were conducted.

The most common macroscopic lesions observed were congenital renal cysts contributing 45.5% of kidneys condemned. Simple congenital renal cysts were common involving 92.0% of the cystic kidneys, while 53.2% had unilateral involvement. Other common lesions observed were non-suppurative interstitial nephritis (39.2%), hydronephrosis (26.6%), congestion (6.4%) and chronic interstitial nephritis (4.7%). A combination of lesions were observed in each kidney. Various degree of severity were observed in non-suppurative interstitial nephritis.

Of 215 sera studied for antibodies against leptospires using Microscopic Agglutation Test, 19.6% were positive. *Leptospira pomona*, *L. tarassovi*, *L. canicola* and *L. pyrogenes* being the common serovars. No leptospiral organisms were cultured and the possible reasons for this failure are briefly discussed.

CONTENTS

INTRODUCTION

The kidney is a vital organ in eliminating the end products of metabolism particularly the nitrogeneous waste products such as urea, creatinine, and ammonia and in maintaining homeostasis by selective excretion of water and solutes. Any disease state through infection, vascular injury, neoplasm or anomaly may exert a compromise on the normal functions of the kidney.

Renal disorders have not received much attention in pig industry. Swine with certain acquired or congenital renal disorders may survive without much clinical manifestations and clinically undetected. For this reason many renal lesions are only observed incidentally in the abattoir during the routine gross post-mortem meat inspection. The abnormal kidney is usually condemned without subjecting it to further laboratory examination to arrive at an accurate diagnosis. Abattoir studies on kidneys of swine have been conducted in many countries but in Malaysia they are almost totally lacking.

Leptospira is one of the aetiologic agents that can cause kidney damage and is considered an economically important disease in the pig. When it is introduced into a susceptible breeding herd foetal losses through abortions and stillbirths may become a major problem in this herd (Hanson, 1981). In Malaysia, an outbreak of abortion in pigs due to Leptospira icterohaemorrhagiae was reported by Brandenberg and Too, 1981. Leptospira is also believed to be an important occupational disease among rice field workers in West Malaysia (Tan, 1970).

2/....

The objectives of this project are therefore to study **firstly** the pathology of kidneys of swine condemned at slaughtered and secondly the prevalence of leptospirosis in slaughtered pigs by cultural and serological examinations.

LITERATURE REVIEW

Several workers have carried out studies on the renal disorders of various species of animal. Tham and Sheikh-Omar, 1981 made a survey on the causes of condemnation of carcasses and organs at Shah Alam abattoir and reported that the main reasons for the rejection of 382 kidneys in pigs were congenital cysts (65.7%), infarcts (13.6%), nephritis (11.0%) and hydronephrosis (4.7%). In a study of 4166 cattle at Dublin abattoir, 4.2% of the kidneys were rejected for gross abnormalities consisting mainly of focal interstitial nephritis (60.1), cysts (26.0%), pigmentation (6.4%) and amyloidosis (2.9%) (Monaghan and Hannan, 1983). An investigation of kidneys of pigs condemned for renal cysts showed that 47.5% of the kidneys belonged to progenies of a Landrace boar, which indicated a genetic origin of the renal cysts in the breed (Wells et al. 1980).

Neoplasms do not represent important causes of condemnation of kidneys at slaughter. In a survey of 3.7 million pigs in abattoirs in Great Britain, Ahderson et al. (1969) found only 16 cases of neoplasm involving the urinary tract, 13 of which were nephroblastomas and 3 others were renal carcinomas. Of 1.3 million cattle examined in the same survey, 11 cases of urinary tract neoplasms were observed consisting of 9 carcinomas, 1 nephroblastoma and 1 pararenal teratoma.

3/....

Jones (1968) reported that cystitis and nephritis were the causes of death in 14.7% of sows studied and that _Corynebacterium_ suis and _E._ coli were two common organisms isolated from affected kidneys. In another survey involving 90 pigs, Wilson _et al._ (1972) observed that 53 (58.0%) had evidence of kidney damage mainly interstitial in nature and predominantly focal presence of lymphocytic.

Leptospirosis in man and rats in Malaysia was first described by Fletcher in 1927. The prevalence of leptospirosis in Malaysia was reported to be 11% in pigs, 28% in goats and 4% in cattle (Smith, 1961). In a 1969 survey conducted by Veterinary Research Institute (VRI) 7.7% of pigs and 8.1% of cattle were found to be sero positive while in a recent (1975-78) survey by VRI, a higher prevalance was seen in the pigs at 23.0%, in cattle at 35.3%, in goats at 7.7% and in dogs at 55.5% while all sheep were seronegative (Joseph, 1979). In a survey conducted in Kluang, Arunasalam (1975) revealed that the infection rate in 163 cattle was 30.67% with serovars _L. pomona_, _L. hebdomadis_, and _L. hyos_ the most common.

MATERIALS AND METHODS

Three hundred and forty two kidneys of 252 pigs condemned at routine meat inspection, were collected on scheduled visits at Shah Alam abattoir from 6th. June 1983 to 17th. June 1983.

The kidneys were put in individual plastic bags, and kept in the cold room at Universiti Pertanian Malaysia for 10 to 13 hours before a detail examination was conducted the next morning.

4/....

Pathology:

The kidneys were examined grossly on the external and cut surfaces and gross abnormalities were noted. Sections from an area with pathological changes were taken and fixed in 10% buffered-formalin. Thin sections of these tissues were stained with hematoxylin and eosin and examined under light microscope.

Microbiology:

Samples of kidneys with changes suspected to have been caused by bacteria were taken for bacteriological examination. Each sample was inoculated onto blood agar and incubated at 37°C for 24 hours. Identification of organisms isolated performed.

A total of 70 kidneys samples were collected based on the macroscopic pathological changes suspected to be due to leptospirosis. 0.2 ml of homogenated kidney tissue was cultured into 5 ml of semisolid Johnson-Seiler medium enriched with bovine albumin with the addition of 400 ug/ml of 5-Flurouracil and 200 ug/ml of 5-FU respectively.

Further 0.2 ml of 10-fold dilution of the homogenate was subcultured with the same preparation of media. The bottles of kidney cultures were then incubated at 30°C. Dark field microscopic examination was conducted on the 3rd, 6th, 8th, and 12th week of incubation period. If leptospiral organisms were not observed by the 12th week, that culture was considered to be negative and discarded.

Serology:

Blood samples of 215 pigs were collected randomly as each of them was being slaughtered. Microscopic agglutination test was

5/....

performed as described by Cole *et al*, 1973 using live cultures of 8 serovars; *hardjo, icterohaemorrhagiae, pomona, canicola, tarassovi, pyrogenes, celledoni,* and *australis* grown on Stuart's medium for 7 days at 30°C. The end point in a positive test was in which at least 50% of the leptospires were agglutinated.

RESULTS AND DISCUSSION

A total of 252 pigs were included in this study. Ninety pigs had bilateral kidney condemnations while the remaining 162 pigs had unilateral condemnations giving a total condemnation of 342 kidneys. Gross and microscopic lesions are presented in Table 1.

Congenital renal cysts were the most common macroscopic lesion observed contributing 154 of kidneys (45.5%) in 144 pigs (57.1%). The size of the cyst varies from 0.2 mm to 6 cm in diameter (Figure 1) Several cysts were just visible externally but a few were only observed on the cut surface. Pressure necrosis of parenchymal tissue were seen in some cases associated with developing cysts. Most of the cysts were located in the cortex.

Of the 154 kidneys with cysts, 142 (92.0%) were simple cysts present in fewer number but larger in size. The remaining 12 kidneys were polycystic as they were numerous but smaller cysts. (Jubb and Kennedy, 1970).

Unilateral cystic kidneys were common involving 134 pigs (53.2%) as compared to bilateral cystic kidneys involving 10 pigs (3.9%).

6/....

Simple congenital cysts are common in kidneys of pigs as reported in literature (Jubb and Kennedy, 1970; Smith and Jones, 1972) they however seldom the causes of renal failure and the affected pigs would survive to adulthood and the lesion always found incidentally at slaughter. Polycystic kidney in swine and other animals may be associated with cysts in other organs but the causes responsible for the syndrome is unknown (Webster and Summer, 1978).

Wells et al. 1980 proved that renal cyst in Landrace is genetically determined. In man, kidney infantile polycystic kidney disease is inherited through an autosomal recessive gene and adult polycystic kidney is inherited through an autosomal dominant gene (Ryan, 1981).

Seventy six (30.2%) of the pigs studied had hydronephrosis, 61 (80.3%) of which were unilaterally affected while the remaining 15 (19.7%) pigs were bilaterally affected. The severity of hydronephrosis range from mild to severe. Progressive dilation of the pelvis and blunting of the apices of the pyramids were observed in mild hydronephrosis. Severe unilateral hydronephrosis were observed in 3 pigs which were flattened with larger outside diamension and tranformed into thin walled sac (Figure 2). Severe hydronephrosis always has unilateral involvement with the opposite kidney is usually normal or hypertrophied, allowing the animal to survive. There are several causes leading to hydronephrosis. In pigs, atresia or kinking of the ureter and obstructive urolithiasis are the frequent causes (Monlux and Munlux, 1965).

7/....

Table 1

Gross and microscopic lesions* observed

in the condemned kidneys of 252 pigs

Lesion	Number of pigs			Total number kidneys (%)
	Unilateral involvement	Bilateral involvement	Total	
Congenital cyst	134	10	144	154 (45.5)
Non-suppurative interstitial nephritis	40	47	87	134 (39.2)
Hydronephrosis	61	15	76	91 (26.6)
Congestion	10	6	16	22 (6.4)
Chronic interstitial nephritis	6	5	11	16 (4.7)
Lobulation	5	2	7	9 (2.6)
Haemorrhage	4	1	5	6 (1.8)
Intravascular coalution	2	2	4	6 (1.8)
Glomerulonephritis	0	2	2	4 (1.2)
Abscessation	1	0	1	1 (0.3)
Total no. of pigs with condemned kidneys	162	90	252	342**

a combination of lesions in each kidney were observed

*total number of kidneys condemned and examined

Three pairs of kidneys were grossly congested. The cause of these congested kidneys remains unestablished but toxemia such as colisepticemia has been suggested as one in younger animals (Jubb and Kennedy, 1970).

Chronic interstitial nephritis is frequently seen in pigs (Monlux and Monlux, 1965). In this study it was seen in 16 kidneys in 11 pigs, occuring bilaterally in 6 pigs and unilaterally in 5 pigs. Grossly, the affected kidneys appeared pale focally or diffusely, and were firmer than normal and had irregular surface (Figure 3). Microscopic appearance was that of marked fibrosis in the cortex and medulla with periglomerular fibrosis, dilatation of the tubules some containing protein cast while others had become atrophied or disappeared and replaced by fibrous tissue (Figure 4).

Foetal lobulations were observed in 7 pigs, 5 unilaterally and 2 bilaterally (Figure 5). This is not a significant finding and is due to the failure of fusion of individual renal anlagen (Jubb and Kennedy, 1970).

The kidneys from 5 pigs, 4 unilaterally and 1 bilaterally showed mild haemorrhages mainly in the renal cortex in association with diffuse non-suppurative interstitial nephritis. Petechiations were visible grossly on the capsular and cut surfaces of the cortex. In case of diffuse interstitial nephritis, the haemorrhage appeared as cortical ecchymoses and papillary petechiation.

Another type of circulatory disturbance commonly seen in the study was intravascular coagulation as was observed in 4 pigs, 2 bilaterally and 2 unilaterally affecting mainly the capillaries in the medulla.

Bilateral subacute proliferative glomerulonephritis was seen in 2 pigs microscopically as hypercellularity of the glomerular tufts filling the Bowmen's space and adhering to the parietal layer (Figure 6). Glomeruli also appeared swollen as the glomeruli tuft increased in size. The affected Bowmen's capsule was slightly thickened. Grossly, there were petechiations in the cortex of the affected kidneys. It is believed that immunologic mechanisms are involved in this disease (Smith and Jones, 1972).

Only one case of renal abscessation observed in this study. It was 2 cm in diameter located in the middle of the kidney involving both the cortex and medulla. Corynebacterium pyogenes and E. coli were isolated from swabs of the abscess.

Of 252 pigs studied, 87 (34.5%) pigs had non-suppurative interstitial nephritis with a severity ranging from slight to severe. Out of 87 pigs which had this condition, 47 (54.0%) pigs were bilaterally affected while the remaining were unilaterally affected. Yellowish foci were observe grossly on the surface and sometimes on the cut surface in case of mild interstitial nephritis. The number of foci increased with increase in the severity. Petechiations were often observed in the cortex of affected kidneys. In severe cases a few ecchymoses and a large number of petechiations in the cortex. These kidneys were also marked pale, enlarged, swollen, oedematous and soft in consistancy (Figure 7).

Table 2

Degree of severity of non-suppurative interstitial nephritis observed in kidneys of 87 pigs

Score*	Unilateral	Bilateral	Total (%)
1+	28	19	47 (54.0)
2+	3	11	14 (16.1)
3+	5	7	12 (13.8)
4+	3	3	6 (6.9)
5+	1	7	8 (9.2)
Total (%)	40 (45.9)	47 (54.0)	87 (100)

* 1+ = few foci (1 to 2 lymphocytic foci)

2+ = several foci (3 to 5 lymphocytic foci)

3+ = many foci (6 to 10 lymphocytic foci)

4+ = numerous foci

5+ = diffuse

Microscopicly, the main characteristic of the non-suppurative interstitial nephritis were the presence of large numbers of lymphocytes and a few plasma cells in the interstitium accompanied by variable degree of tubular atrophy (Figure 8). Based on the number of the lymphocytic foci the condition was graded to 1+, 2+, 3+, 4+ and 5+ (Table 2). Focal non-suppurative interstitial nephritis with 1+, 2+ and 3+ scores were more common (83.8%) while the diffuse type was uncommon. In calves, focal non-suppurative insterstitial nephritis is known as 'white-spotted kidney' has unestablished causes and pathogenesis. In swine, focal interstitial nephritis occurs as a residue in leptospirosis (Jubb and Kennedy, 1970). In the study we failed to demonstrate leptospiral organisms using silver stain in the kidney sections which exhibited non-suppurative interstitial nephritis.

A total of 74 (21.6%) kidneys had more than one lesion observed during microscopic examination. The mixed lesions are tabulated in Table 3. The mixed lesions of cyst and hydronephrosis represented the most common mixed lesions involving 36 (10.5%) of the total kidneys surveyed. However, the condemnation of a particular kidney was due to the most obvious gross lesion like cyst, hydronephrosis or due to lobulation. The condemnation of remaining 268 (78.4%) kidneys were due to one lesion appeared macroscopically.

A total of 42 (19.6%) of the sera reacted to one or more leptospira serovars while 4 sera (1.9%) reacted to two serovars. The prevalence of leptospirosis observed is quite low compared to that of

Table 3

Mixed lesions observed in the condemned kidneys of 252 pigs

Mixed lesion	No. of cases: Unilateral involvement	No. of cases: Bilateral involvement	Total no. of kidneys (%)
a + b	26	5	36 (10.5)
a + c	5	2	9 (2.6)
a + d	2	2	6 (1.7)
a + c + d	2	1	4 (1.2)
a + c + e	1	0	1 (0.3)
a + b + c	1	0	1 (0.3)
a + g + h	1	0	1 (0.3)
a + c + d + f	1	0	1 (0.3)
b + c	4	1	6 (1.7)
c + d	2	2	6 (1.7)
c + f	1	0	1 (0.3)
c + f + i	1	0	1 (0.3)
c + d + f	1	0	1 (0.3)
Total no. of pigs with condemned kidneys	162	90	342•

•Total no. of kidney examined

a: congenital cyst
b: hydronephrosis
c: non-suppurative interstitial nephritis
d: congestion
e: abscessation
f: haemorrhage
g: lobulation
h: chronic interstitial nephritis
i: intravascular coagulation

26.6% reported by Bahaman and Dawam , 1984 and 23.0% reported by Joseph, 1979. This low prevalence is likely due to various factors such as low quality of stock cultures used or the stock cultures used were too dilute.

That the most important serovar was serovar pomona (13.5%) is constant with the findings in many other countries and previous reports in Malaysia. Other common serovars observed were L. tarassovi, L. canicola and L. pyrogenes. The serovar L. tarassovi is also commonly associated with swine leptospirosis in many other countries (Hanson, 1981). Four sera reacted to two serovars. These 4 reactors could have had previous multiple infection or spontaneous agglutination of the antigen or due to cross-reaction with agglutinins of the other serovars (Bahaman, pers. comm.).

The failure to culture any leptospiral organisms could be likely due to several factors such as the fastidious requirements of the leptospiral organisms, the small number of the organisms in the tissue sample used, the toxic effects of the tissue and cultural contamination from other organisms in the samples.

Leptospires are slow-growing organisms having a generation time of approximately 20 to 24 hours at 30^{o}C as compared to the generation time for bacteria of 20 to 30 minutes (Bahaman, per. comm.). The combination of this factor and the requirement for a rich medium at a natural pH predispore the cultivation of leptospira to contamination arising from the equipment used, the environment or from organisms in the samples.

Table 4

Distribution of leptospiral antibody to 8 leptospira serovar antigens in 215 porcine sera

Serovar	No. positive*	Percentage
pomona	29	13.5
tarassovi	3	1.4
canicola	2	0.9
pyrogenes	1	0.5
cellodoni	1	0.5
hardjo	1	0.5
icteroheamorrhagiea	1	0.5
australis	0	0.0
pomona + canicola	1	0.5
tarassovi + pyrogenes	1	0.5
canicola + pyrogenes	1	0.5
pomona + pyrogenes	1	0.5
Total no. of reactors	42	19.6

*50% of the leptospires were agglutinated

Time may be another factor resulting in the failure to culture leptospires. The kidney tissues collected were processed for culture 10 to 13 hours after having been kept in the coldroom in addition to the initial 4 hours spent during the slaughtering process. Post-mortem changes in pH and drying of the tissue can kill leptospires since they can only survive in a fairly narrow pH range near 7 and cannot withstand drying (Hanson, 1981). The organisms may be lost or reduced in number to a level below the threshold of sensitivity of the cultural technique. Lysis of leptospires by lipids from renal tissue may also be a factor contributing to the early death of the leptospira (Ho and Blackmore, 1979).

A further investigation to improve the sensitivity of the media and adequate facilities to avoid bacterial contamination should be carried out as the aid in diagnosing leptospirosis.

CONCLUSION

The common lesions of the kidneys observed in the study were simple congenital cysts followed by non-suppurative instertitial nephritis and hydronephrosis. Most kidneys had more than one lesion.

The prevalence of the leptospirosis was low at 19.6%. most common serovars observed were L. pomona, L. tarassovi, L. canicola and L. pyrogenes. Leptospiral organisms were difficult to culture because they were slow growing organisms, survive in narrow pH range and unable to withstand drying.

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Fig. 1: Simple Renal cyst.

Fig. 2: Severe hydronephrosis.

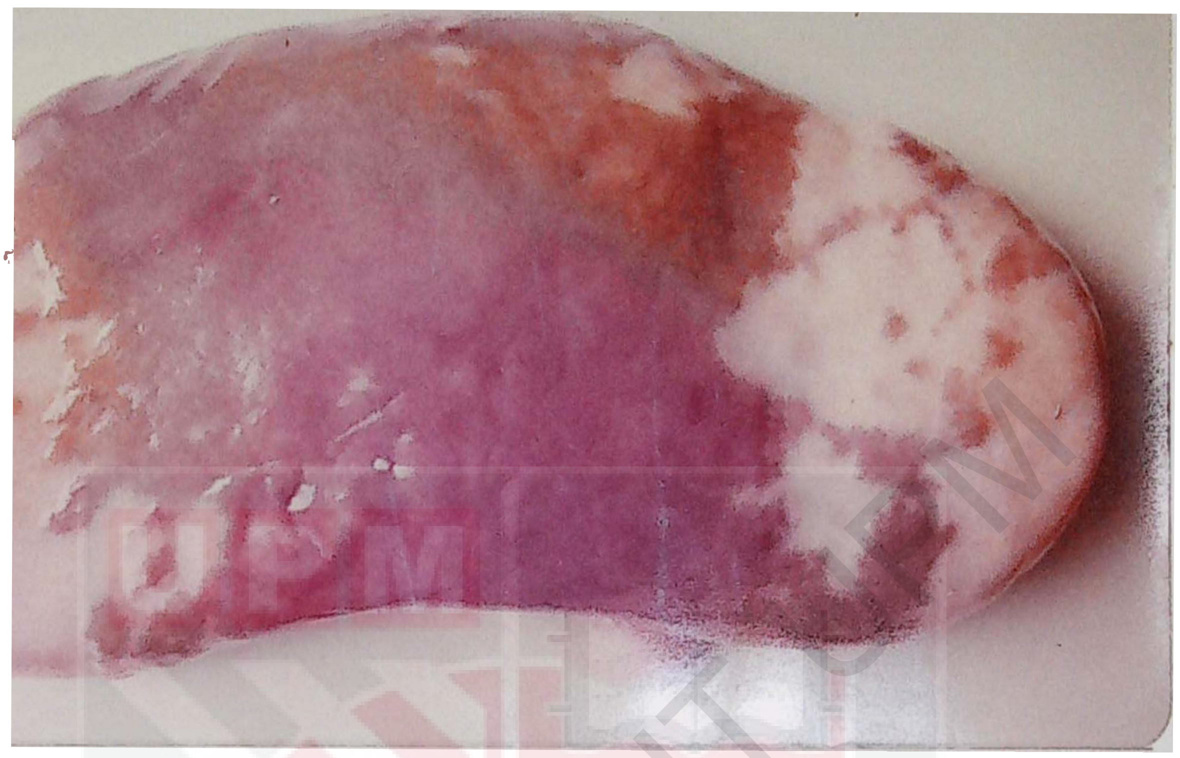

Fig. 3: Chronic interstitial nephritis.

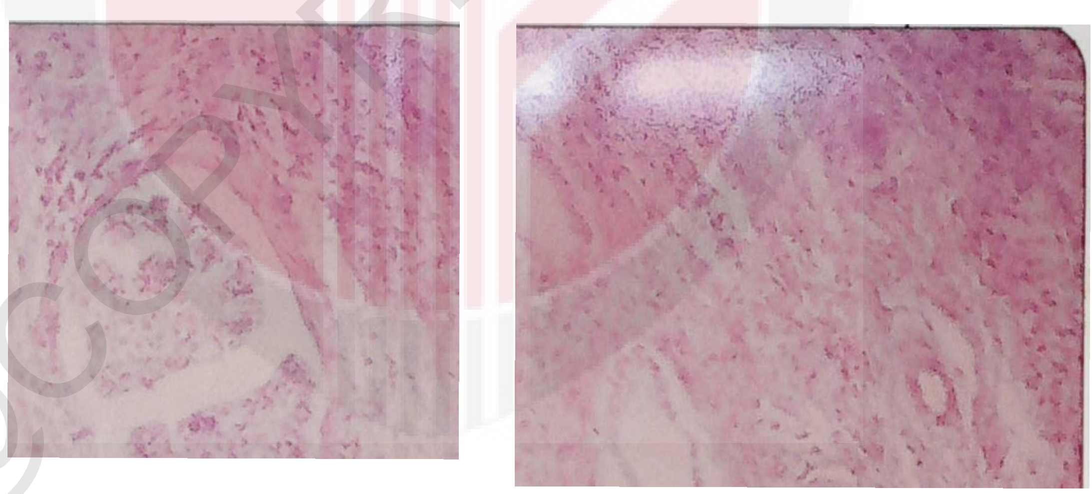

Fig. 4: Chronic interstitial nephritis (Microscopic)

Fig. 5: Foetal lobulation

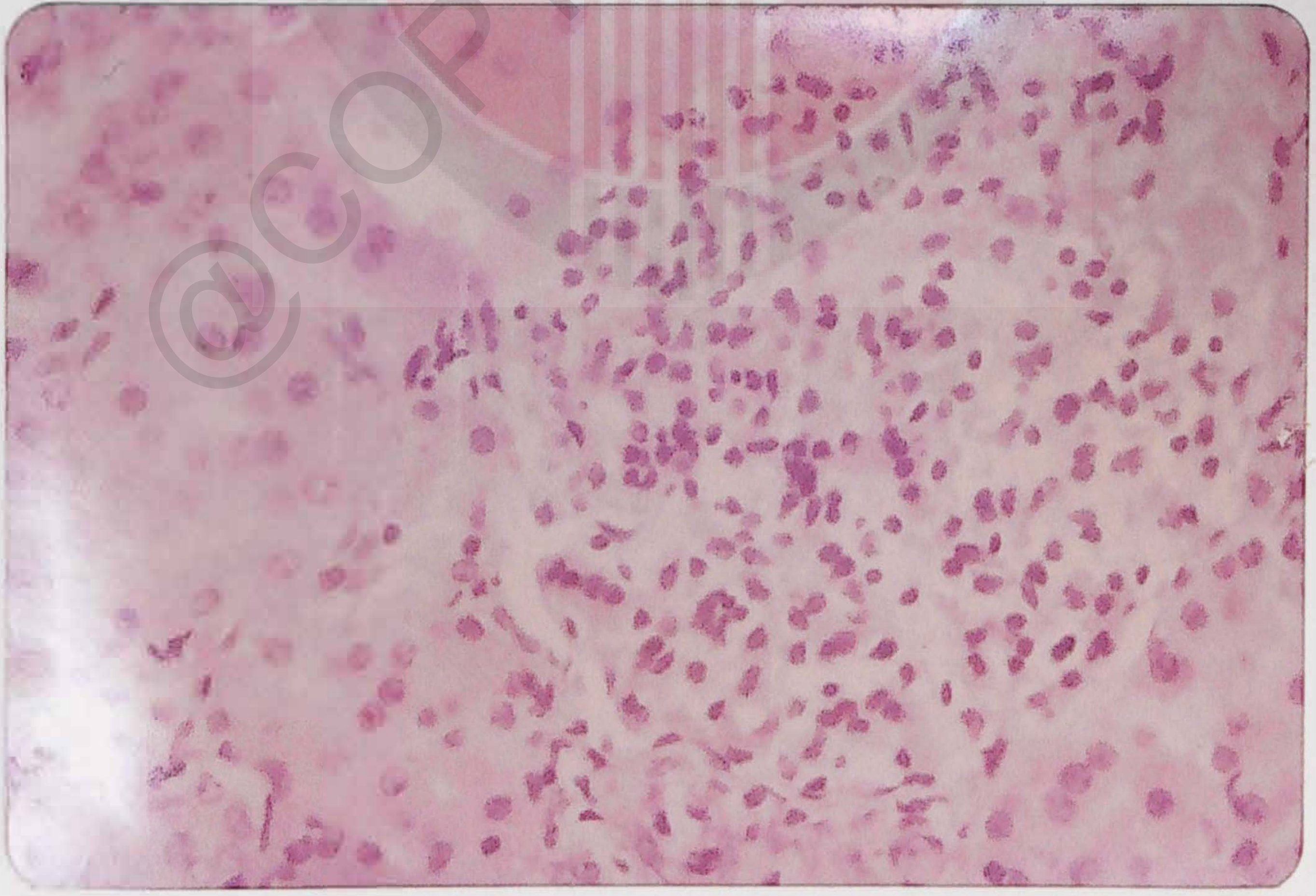

Fig. 6: Proliferative glomerulonephritis (Microscopic)

Fig. 7: Diffuse non-suppurative interstitial nephritis.

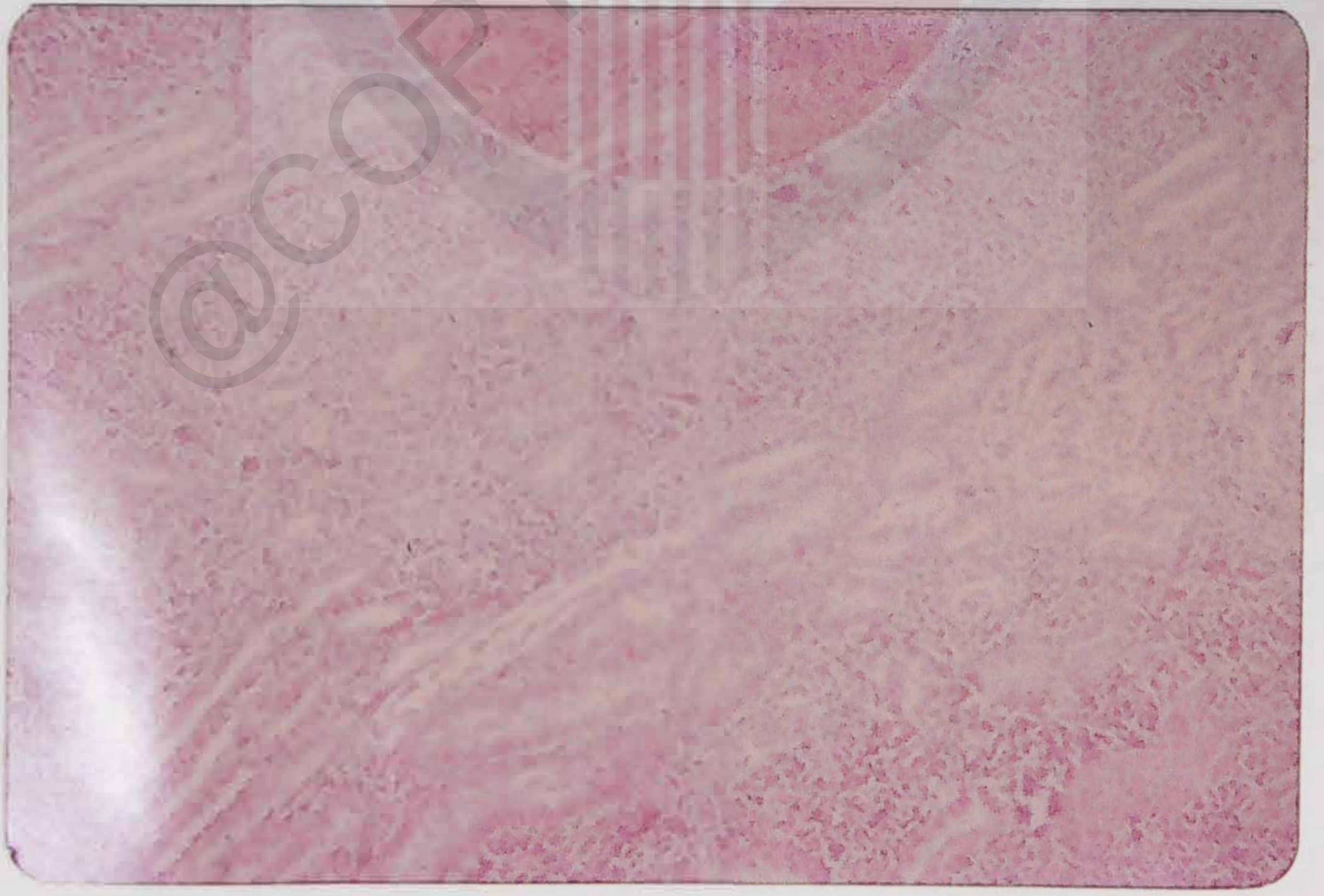

Fig. 8: Diffuse non-suppurative interstitial nephritis (Microscopic)