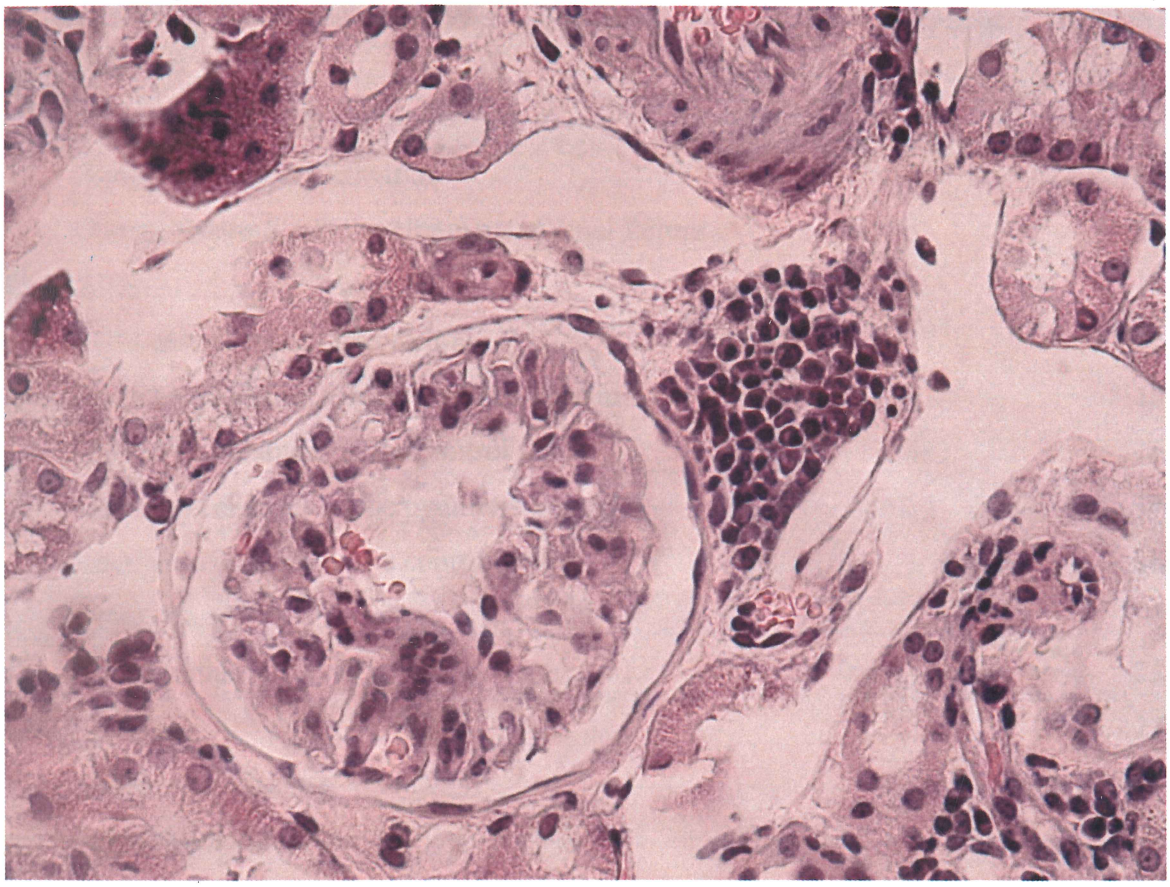
RENAL DAMAGE IN DOGS WITH PYOMETRA COMPARED TO AGE MATCHED CONTROL DOGS: MICROSCOPIC GLOMERULAR AND INTERSTITIAL CHANGES, AND DEVELOPMENT OF CLINICAL RENAL FAILURE



Fordypningsoppgave for

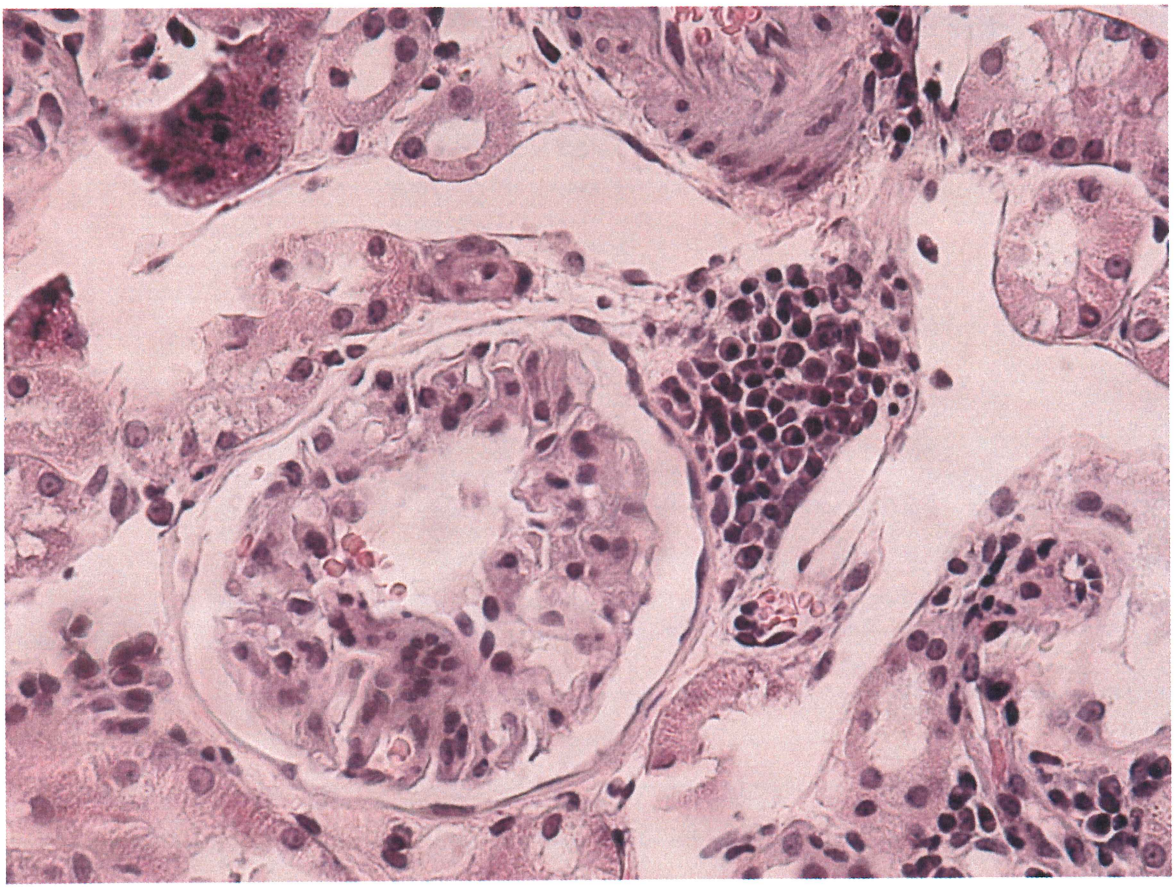
Tone Madsen og Veronica Mikalsen, kull 97

Norges veterinærhøgskole, 2003

Veileder: Reidun Heiene

Institutt for smådyrsjukdommer

RENAL DAMAGE IN DOGS WITH PYOMETRA COMPARED TO AGE MATCHED CONTROL DOGS: MICROSCOPIC GLOMERULAR AND INTERSTITIAL CHANGES, AND DEVELOPMENT OF CLINICAL RENAL FAILURE



Fordypningsoppgave for

Tone Madsen og Veronica Mikalsen, kull 97

Norges veterinærhøgskole, 2003

Veileder: Reidun Heiene

Institutt for smådyrsjukdommer

INDEX

ABSTRACT

There is controversy concerning investigations done in dogs with pyometra. The aims of the study was to examine whether pyometra dogs have renal lesions beyond age-related changes found in this particular age group, and to find whether the potential for persisting renal disease increases in dogs with pyometra.

Kidney biopsies from 19 dogs with pyometra in a previous study, were examined and classified by light microscopy, and compared to corresponding types of changes in 16 age matched control dogs. The control dogs were euthanized or dead due to conditions without a known internal medicine component. The main focus in the comparison of the two groups was glomerular and interstitial changes.

Prevalence of clinical renal failure later in life in 40 dogs with pyometra was investigated in a questionnaire. The owners were interviewed approximately eight years after surgery about clinical signs of renal failure, and about the cause of death or euthanazia in their dog.

Histopathological examination revealed more pronounced glomerular changes (glomerulonephritic and glomerulosclerotic lesions) in the control dogs compared to the pyometra dogs. Severe interstitial infiltration of mononuclear cells was found only in the dogs with pyometra.

Of the 41 dogs included in the retrospective questionnaire, five had been drinking more than usual after surgery and towards the end of the dog's life. Twenty-four dogs had been euthanized or died due to conditions apparently not related to renal failure. The cause of death/euthanasia was poorly defined in 11 dogs. Two dogs had been euthanized due to

confirmed renal failure. These dogs also had severe proteinuria at the time of surgery in the original study. Four dogs were still alive and in good shape.

To conclude, glomerular damage beyond age related changes could not be demonstrated by light microscopy in these dogs with pyometra. Age-related renal changes in the dog include glomerular lesions. Proteinuria may persist in patients after ovariehysterectomy and predispose for the development of renal failure.

INTRODUCTION

Important questions concerning renal damage in dogs with pyometra, and the prognosis related to this disease, are still unsolved in veterinary medicine. The background for this study is the controversy of whether dogs with pyometra develop secondary chronic renal damage or not. Development of glomerulonephritis in pyometra dogs is a generally accepted hypothesis and has become a "clinical truth", in despite of inadequate support of research data. There are a few reports done on renal morphology in pyometra dogs, but the evidence is sometimes conflicting.

The hypothesis of glomerulonephritis secondary to pyometra is based on a study[9] on 27 pyometra dogs. Glomerular changes under light microscope corresponded to the changes of membranous or mixed membranous and proliferative glomerulonephritis in human beings. The study had a control group of only five young (1-6 years) dogs.

In an earlier study, no glomerular changes were found in a group of pyometra dogs.[1] This study also concludes that the renal interstitial changes seen in many pyometra dogs represents a concomitant acute or sub acute interstitial nephritis, which has nothing to do with the uterus lesion. This study had no control group at all.

In 1988, a study[12] was done on 27 pyometra bitches, controlled by 12 healthy female dogs. The study assumed that glomerular changes in these dogs most likely were age-related, because no difference between pyometra dogs and the age-matched control group were revealed. This assumption was based on a comprehensive work that included immunohistochemistry, electron microscopy and light microscopy.

To draw any conclusion from renal morphology in a group of pyometra dogs, an age-matched control group has to be included in the study to eliminate normal age-related renal changes from possible pyometra-induced ones. Although old dogs are reported to have chronic renal lesions of miscellaneous and often unknown aetiology,[3] [12] there is very little information available on spontaneous renal ageing in normal dogs. One study[3] refers to unpublished data, when claiming that nephrosclerosis of varying severity is a common morphologic abnormality of geriatric dogs, and that it is frequently recognized in the absence of clinical or biochemical evidence of renal insufficiency. There are few other studies concerning this subject.[3,4] So far, most of the knowledge about age-related renal changes in dogs are based on literature concerning humans and rodents.

In a previous investigation on pyometra dogs,[5] serious renal changes were found by light microscopy in some of the bitches. In the absence of an age-matched control group, it was difficult to know if these changes were related to pyometra, and whether they indicated permanent renal lesions or not. To solve this problem, the present study was initiated. An age-matched control group for retrospective comparison to the pyometra dogs, was established, and the same pyometradogs were traced to find if signs of clinical renal failure after the surgery had been noticed by the owners.

The aims of the present study are:

1. To examine whether glomerular and interstitial renal changes in pyometra bitches, differs light microscopically from renal changes in a control group of age-matched dogs.
2. To find the prevalence of later development of clinical renal failure in dogs treated for pyometra.

MATERIAL AND METHODS

Pyometra dogs (group P)

Renal biopsies from 19 dogs with pyometra were studied by lightmicroscopy. The dogs were randomly chosen from a material consisting of 55 pyometradogs in a previous study (Heiene, 2001). The average age in this selected group is 8.7 years, ranging from 7 to 14 years. Dogs younger than 7 years were excluded. The pyometra dogs are presented in Table 1. In dog P2 and dog P12 the exact age is not known (not mentioned in the journal).

Control dogs (group C* and C)

Renal tissue from three old dogs euthanized at external clinics (group C*) for other reasons than internal medicine conditions were examined by light microscopy. The control group also consisted of 13 dogs supplied by a post mortem material from department of pathology at the veterinary school of science (group C). These dogs were selected by searching journals from routine autopsy material gathered the last five years (1997-2002). Relevant journals were found, and were further traced back to the veterinarian who submitted the dog for autopsy, to rule out that the dog died of any known internal medicine condition. The control group consists of 16 elderly dogs with an average age of 9,8 years ranging from 7 to 13 years. The breed is unknown in dog C2 (not mentioned in the journal).

The inclusion of both subgroups of control dogs was based on the following criteria: female dogs older than seven years, euthanized or dead for other reasons than known internal medicine conditions (behavioural, skeletal problems etc.). The owners of the dogs from the clinics (group C*) confirmed that their dog had never shown signs of PU/PD, infection in the urinary tract, weight loss, reduced general condition lately, long lasting or severe illnesses earlier (See Appendix 1). The control dogs are presented in Table 1.

Table 1: Presentation of the dogs

P=pyometra dogs

C=control dogs from post mortem material

C*=control dogs from the clinics

Pyometra dogs		
no.	**age**	**breed**
P1	7	New Foundland dog
P2	?	Kleiner munst.
P3	10	Golden Retriever
P4	9	Labrador
P5	9	Mixed breed
P6	7	New Foundland
P7	7	English Setter
P8	7	Borzoi
P9	10	Golden Retriever
P10	11	Mixed breed
P11	7	Miniaturepoodle
P12	?	Golden Retriever
P13	7	Schäfer
P14	7	Vorsteher
P15	8	St. Bernhard
P16	10	Borzoi
P17	10	Golden Retriever
P18	14	Alaskan Malamute.
P19	7	Boxer

Control dogs			
no.	**age**	**breed**	**cause of death**
C1*	13		Old
C2	11		Gastric torsion
C3	8	Golden Retriever	Trauma
C4	9	?	Hipdysplacia
C5	9	Vorsteher	Gastric torsion
C6	10	English Setter	Trauma
C7	9	Doberman	Gastric torsion
C8	8	Flat Coat Retriever	Spinalcord degeneration
C9	12	Bichon Frisée	Corpus alienum, bowel
C10	11	English Setter	Old
C11	8	Irish Terrier	Bleeding in the spine.
C12	13	Mixed breed	?
C13	9	Bernease mount.	Gastric torsion
C14	12	Breton	Trauma
C15*	7	Italian mynde	Old
C16*	8	Leonberger	Old

Biopsies

Renal biopsies from the pyometradogs were obtained during surgery in a previous study[5]. Two biopsies were taken from the cortical part of the right kidney with a Bard Biopty biopsy instrument (BardLtd. England). The biopsies measured 1-2 mm in diameter and were maximally 15 mm long.

Pathology methods

Renal tissue from the dogs in group C was taken by the pathologists as part of the routine autopsy program at department of pathology at the Norwegian School of Veterinary Science (1997-2002). Renal tissue from the dogs in group C* was obtained within 6 hours post mortem (2002-2003). In each dog one section was removed with a scalpel from either the left or the right kidney, including both cortex and marrow. The sections measured 1-2 cm in diameter and were 2-3 cm long. All the dogs had been euthanized by Pentobarbital.

Kidney biopsies and renal sections were fixed in 4% formaldehyd immediately after the surgery or autopsy procedure. The specimens were then prepared for standard histological examination by the technicians at the department of pathology at the Veterinary School of Science. The preparation included staining with heamatoxylin and eosin (H&E), and van Gieson.

Histopathological Evaluation

All the biopsies and slices were examined by light microscopy. Due to post mortem changes in tubuli in the post mortem material, only glomerular and interstitial changes were evaluated.

At least five glomeruli per specimen had to be present for the specimen to be valid. A systematically evaluation was first done by following a pre-made protocol (Appendix 2) consisting all the glomerular and interstitial components to be studied. This protocol was used to assure that no changes were overlooked, and its content was based on knowledge and terminology from standard histopathology textbooks. First a specific grading were done with emphasis on the individual changes in the specimen, then a total evaluation in each dog were done based on this grading and the general impression of the specimen as a whole.

The different kinds of changes focused on, in the specific grading, were the degree of sclerosis, amount of spindle-shaped cells and thickened Bowman´s capsules when it comes to the glomeruli. In the interstitium, the degree of sclerosis and mononuclear cell infiltration was the main focus.

The grading system included; normal findings (0), slight degree (+), moderate degree (++) and severe degree (+++) of changes. The aim of this specific grading was to lighten the relative differences and to reveal the background for the later total evaluation.

A total evaluation of changes was done in each dog. Glomerular changes and interstitial changes were graded separately, and following the same grading system as described above. Afterwards an experienced pathologist did a blinded grading.

Glomerular changes

The glomeruli were evaluated in regard to hypercellularity, especially the richness and distribution of spindle shaped cells were noted. Infiltration of polymorph nucleated and mononuclear cells were also registered. The degree of hyalinisation in the glomerular tufts (H&E) and the degree of sclerosis verified by van Giesen staining was registered as well. Thickening of the basement membrane was also noted, but was only based on a general impression, since no special staining was done for this purpose.

Category: Normal

Within the category of normal kidneys, a very slight degree of changes was accepted (fig. 3). Observations of just few spindle shaped cells in one or another glomeruli, one or two segmental sclerotic glomeruli or with a thickened Bowman's capsule were tolerated as well.

Category: Slight degree

The criteria for grading glomerular changes as slight degree are the presence of some spindle shaped cells diffusely distributed throughout the glomeruli. The presence of barely viewable sclerotic areas scattered in the mesangium, verified by van Gieson staining (fig.4) and presence of one and another thickened Bowman's capsule will also satisfy this category. In the total evaluation of each section, the amount of affected glomeruli and the degree of sclerosis is taken into account.

Category: Moderate degree

As the degree of glomerular changes increases from slight to moderate, hypercellularity with presence of several spindle shaped cells in the glomerular tufts has to be present (fig.5). The sclerotic areas have to be more pronounced with segmental distribution in several glomeruli. The capillary lumen seems to be narrowed in glomeruli satisfying the category of moderate

degree of changes, and thickened Bowman's capsule might affect several glomeruli in the specimen.

In the total evaluation the degrees of glomerular changes did not have to be uniform. Different degrees of changes might be observed in different glomeruli in the same section and still satisfy the category of moderate degree of changes.

Category: Severe degree

The severe degree of changes (fig. 6) is in this study defined as glomeruli with a large number of spindle shaped cells in many glomeruli. Sclerosis, verified by van Gieson staining has to affect most of the glomeruli and global distribution might be seen in several glomeruli.

Interstitial changes

The renal interstitium was evaluated in regard to the degree of infiltration and distribution of mononuclear cells. The amount and distribution of spindle shaped cells were also noted. The red collagenous reaction visible by van Gieson staining was focused on as a sign of sclerosis/fibrosation of the interstitium.

Category: Normal

Within the category of normal, very slight degree of changes is tolerated (fig. 3). Observations of just a few mononuclear cell infiltrations in parts of the interstitium and a minor presence of sclerosis were accepted as normal findings.

Category: Slight degree

The criteria for grading interstitial changes as slight degree are the presence of some mononuclear cell infiltration diffusely distributed in the interstitium (fig. 7). The presence of barely viewable sclerotic areas, verified by van Gieson staining, also satisfies this category.

Category: Moderate degree

To grade the interstitial changes as moderate degree, the mononuclear cell infiltration has to dominate the specimen by forming clusters diffusely scattered all over the interstitium (fig. 8). Pronounced sclerotic areas distributed in larger regions or plain strains would also satisfy this category.

Category: Severe degree

The severe degree of changes in this study is defined as extensive distribution of mononuclear cell infiltration. The presence of larger regions of sclerosis that replace parts of the tubuli in the interstitium would also satisfy this category.

Questionnaire: Later development of clinical renal failure in dogs with pyometra

41 owners of dogs with pyometra included in a previous study[5] at Norwegian Veterinary School of Science from 1993 to 1994 were interviewed approximately 8 years after surgery about clinical signs and about the cause of death or euthanasia in their dog.

In a telephone interview the owners answered the following questions:

1) Did the dog drink more than usual after the operation? If so, when did it stop?

2) Did the dog drink more than usual throughout the whole life?

3) Is the cause of death known? If so, what was the cause?

4) If the dog died of renal failure, was the diagnosis confirmed (blood test or autopsy)?

5) Was renal failure known at any time after operation?

RESULTS

Light-microscopic observations

The results from the light-microscopic observations in the pyometra- and the control group are presented in Table 2. The incidence of different changes in the two groups is summarized in Table 3.

Glomerular changes in the pyometra group

Total evaluation

Eight (42%) dogs of the material representing the pyometra group, revealed changes in the glomeruli. In general, very varied changes were observed, both in terms of types of changes and whether they were focal or generalized. The summarized evaluation depended on the total impression of each section.

Category: Slight

Six (32%) dogs had changes of the slightest degree, which basically consisted of some spindle shaped cells and thin small hyalinized regions (H&E) with a slight sclerotic appearance (van Gieson) in the glomeruli (fig. 4). Some sections did not reveal other changes than a couple of sclerotic glomeruli, while others just revealed a thickening of the Bowman's capsule of some glomeruli (fig. 8).

Category: Moderate

Two (10%) dogs were graded as having a moderate degree of changes. In these dogs (P10 and P16) the mesangium of the individual glomeruli took up a greater percentage of the total glomerular volume. The sections revealed several glomeruli with plain sclerotic areas visualized by van Gieson -staining (fig. 5).

Category: Severe

No dogs had severe degree of changes.

Specific evaluation

Of all the 19 dogs, 8 (42%) revealed some degree of sclerotic glomeruli (van Gieson), 6 (32%) had some spindle-shaped cells in the glomeruli, and 6 (32%) had thickened Bowman's capsules (Table 3 and Figure 1).

Glomerular changes in the control group

Total evaluation

Twelve (75%) dogs in the control material had changes in the glomeruli. In general the control group revealed more severe changes in the glomeruli than the dogs in pyometra dogs

Category: Slight

Six (38%) of the affected dogs had a slight degree of changes, which basically consisted of some spindle shaped cells and narrow hyalinized regions (H&E) with slight scattered sclerotic appearence (van Gieson) in the glomeruli (fig. 4)

Category: Moderate

Four (25%) dogs revealed a moderate degree, where more spindle-shaped cells and plain, wide hyalinized regions (H&E) with sclerotic appaerence were presented (fig.5). The Bowman's capsule was thickened in all of them. The degree of glomerular changes was seldom uniform. Different degrees of damage could be observed in different glomeruli in the same section.

Category: Severe

Two (13%) of the dogs revealed severe degree of changes, which appeared as abundant spindle shaped cells in the glomeruli. An higher percentage of the total glomerular volume than represented in the sections of moderate degree was observed. Sclerosis verified by van Gieson -staining dominated the picture of the glomeruli (fig. 6).

Specific evaluation

Of all the 16 elderly dogs in this group, 12 (75%) had infiltration of spindle-shaped cells in the glomeruli, 12 (75%) dogs had some degree of sclerosis (van Gieson), and 8 (50%) had thickened Bowman's capsules.

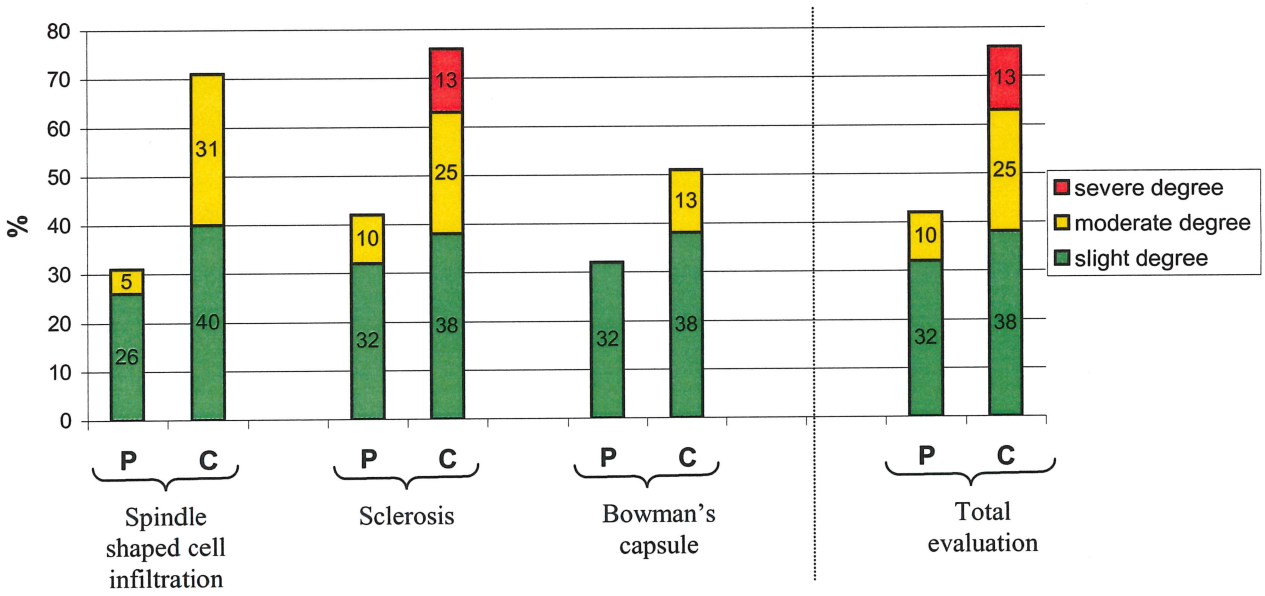


Figure 1: Incidence of different glomerular changes in the pyometra dogs and the control group (P= pyometra dogs, C= control dogs)

Interstitial changes in the pyometra group

Total evaluation

The alterations of the interstitium were dominated of mononuclear cell infiltrations. Most often, the infiltrating cells were clustered about the glomeruli (fig. 7 and 8), but they could also be encountered in small groups around tubules. Eight (42%) of the pyometra dogs had changes in the interstitium. In general, the pyometra dogs revealed more severe changes in the interstitium than the control dogs.

Category: Normal

Within this category, also *very* slight degree of changes was accepted. Usually those changes consisted of small clusters of infiltrating cells scattered in the interstitium. The very slight degree was occasionally observed in category 0 sections, although not enough to justify a change in category.

Category: Slight

Four (21%) revealed a slight degree of changes (fig. 7), which basically consisted of mononuclear cell infiltrations. Only P11 did in addition have some hyalinized strains with collagenous reaction.

Category: Moderate

Four (21%) dogs had a moderate degree of changes (fig. 8), which also consisted of mononuclear cell infiltrations, except dog P16 that revealed plain hyalinized strains with a collagenous reaction in larger regions. There were also observed moderate glomerular changes in this section.

Category: Severe

No dogs had severe interstitial changes

Interstitial changes in the control group

Total evaluation

Only 1 (6%) dog in the control group (C7) revealed substantial changes in the interstitium and was graded as having moderate degree of changes. There were large sclerotic regions (van Gieson) and focal mononuclear cell infiltrations (fig. 8). In contrast to the mononuclear distribution pattern in the pyometra material, the mononuclear cells were clustered in small groups scattered throughout the interstitium. This dog did also show moderate changes in the glomeruli. None of the dogs in the control group had severe interstitial changes.

Specific evaluation

Ten dogs (63%) revealed a very small degree of sclerosis in the interstitium, but not enough to justify classification as slight degree in the total evaluation of the sections.

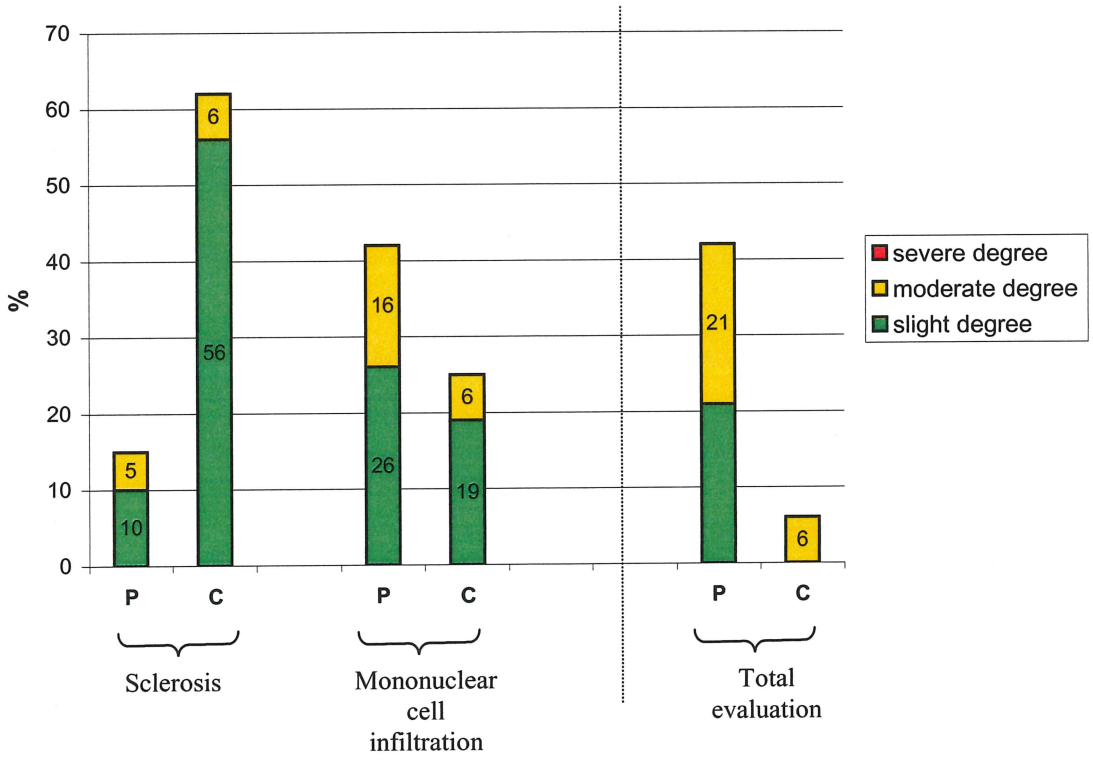


Figure 2: Incidence of different interstitial changes in the pyometra dogs and the control group (P= pyometra dogs, C= control dogs)

Table 2: Survey of the degree of renal changes in pyometra- and control dogs

0 = normal findings
+ = slight degree
++ = moderate degree
+++ = severe degree

		Glomerular changes				Interstitial changes			
	No.	Spindle-shaped cell infiltration	Sclero-sis	Thickened Bowman's capsule	**Total evaluation**	Sclero-sis	Mononucleated cell infiltration	**Total evaluation**	No. (fig.)
Pyometra dogs	P1	+	0	0	0	0	0	0	P1
	P2	0	0	0	0	0	++	++	P2
	P3	0	0	+	0	0	0	0	P3
	P4	0	0	0	0	0	+	+	P4
	P5	+	+	0	+	0	+	0	P5
	P6	0	0	0	0	0	++	++	P6
	P7	0	0	0	0	0	+	+	P7
	P8	0	+	+	+	0	++	++	P8 (fig. 8)
	P9	0	0	0	0	0	0	0	P9
	P10	++	++	+	++	+	0	0	P10
	P11	+	+	0	+	+	+	+	P11 (fig. 7)
	P12	+	+	0	+	0	0	0	P12
	P13	0	0	0	0	0	0	0	P13
	P14	0	0	0	0	0	0	0	P14
	P15	0	+	+	+	0	+	+	P15
	P16	+	++	0	++	++	0	++	P16
	P17	0	+	0	+	0	0	0	P17
	P18	0	0	+	0	0	0	0	P18
	P19	0	0	+	0	0	0	0	P19
Control dogs	C1*	++	++	+	++	+	0	0	C1*
	C2	+	++	+	++	+	0	0	C2
	C3	++	+++	++	+++	+	0	0	C3
	C4	+	0	0	0	+	0	0	C4
	C5	+	+	+	+	0	0	0	C5
	C6	++	+++	+	+++	0	0	0	C6
	C7	++	++	++	++	++	++	++	C7 (fig. 6)
	C8	0	0	0	0	0	0	0	C8
	C9	+	++	+	++	+	0	0	C9 (fig. 5)
	C10	+	+	0	+	+	0	0	C10
	C11	++	+	0	+	+	0	0	C11
	C12	+	+	0	+	+	0	0	C12
	C13	+	+	0	+	+	0	0	C13 (fig. 4)
	C14	0	0	0	0	0	0	0	C14
	C15*	0	0	0	0	0	0	0	C15* (fig 3)
	C16*	0	+	+	+	0	0	0	C16*

Table 3: Incidence of different renal changes in pyometra- and control dogs

		Glomerular changes				Interstitial changes		
	Degree of changes	Spindle shaped cells	Sclero-sis	Thickened Bowman's capsule	**Total evaluation**	Sclero-sis	Mononucleated cell infiltration	**Total evaluation**
Pyometra dogs	slight	5 (26%)	6 (32%)	6 (32%)	**6 (32%)**	2 (10%)	5 (26%)	**4 (21%)**
	moderate	1 (5%)	2 (10%)	0	**2 (10%)**	1 (5%)	3 (16%)	**4 (21%)**
	severe	0	0	0	**0**	0	0	**0**
	any change	*6 (32%)*	*8 (42%)*	*6 (32%)*	***8 (42%)***	*3 (16%)*	*8 (42%)*	***8 (42%)***
Control dogs	slight	7 (40%)	6 (38%)	6 (38%)	**6 (38%)**	9 (56%)	0	**0**
	moderate	5 (31%)	4 (25%)	2 (13%)	**4 (25%)**	1 (6%)	1 (6%)	**1 (6%)**
	severe	0	2 (13%)	0	**2 (13%)**	0	0	**0**
	any change	*12 (75%)*	*12 (75%)*	*8 (50%)*	***12 (75%)***	*10 (63%)*	*1 (6%)*	***1 (6%)***

Other changes

There were a few synechiae between capillary loops and the Bowman's capsule. In the control material, four glomeruli revealed vascular tufts that were contracted to leave a large capsule space. In some sections (Group C), the glomerular changes ultimately resulted in one or another hyalinized, acellular clump.

Protein rich material and epithelial cells were found in the urinary space in several sections (Group C). No emphasis was placed upon these changes as they were thought result from post mortem changes. Some sections also revealed capillaries crowded with erythrocytes in the glomeruli and the interstitium. Those sections seemed to descend from dogs with gastric torsion or trauma.

Questionnaire

A previous study of pyometra dogs [5] was the basis for the questionnaire. Out of 55 dogs, 41 owners were available for follow up questions concerning clinical signs of renal failure and the cause of death or euthanasia in their dog. The dogs were basically of large and middle-sized breeds and 4 dogs were still alive at the time the questionnaire was performed. Five had been drinking more than usual also after surgery or towards the end of the dogs' life and two dogs had been euthanized due to confirmed renal failure. These two dogs had severe proteinuria after surgery in the original study. Twenty-four dogs had been euthanized or died due to conditions apparently not related to renal failure, and in 11 dogs the cause of death was poorly defined. The owners were usually unsure about the cause of death in their dog. Nine owners thought that their dog died of heart problems, 8 thought that their dog died of cancer, 8 thought that their dogs were "just" old and in 5 dogs the owners meant that the dog died of other reasons than renal failure (epilepsy, seizure, hip dysplasia , etc.).

The age was known in 32 of the dogs that were dead, and the median age was 12 years. The 4 dogs that were still were alive during the questionnaire were at least 10 years, and had no signs of clinical renal failure recognized by the owner. The two dogs that died of renal failure were 11 and 12 years.

DISCUSSION

If the glomerular changes in bitches with pyometra were only age-related, or partially induced by pyometra, one would expect the pyometra group to have equal or higher degree of glomerular changes, compared to a control group. Table 2 reveals that glomerular changes observed by light microscopy in bitches with pyometra are less severe than what observed in the age-matched control dogs. This support the hypothesis that pyometra dogs do not get glomerular changes beyond changes in a non-pyometra population.

The fact that the control dogs even had more severe glomerular changes seems strange. Factors like slight age difference between the two groups (1.1 years), small group sizes and uncertain history of the dogs may explain the differences.

The average age of the dogs representing the control material was 9.8 years and the average age of the dogs representing the pyometra material was 8.7 years. In this study geriatric dogs was defined as >7 years. The definition of geriatric depends on factors like genetics, environment and nutrition. Whether a dog older than 7 years is old is only based on a qualified guess, because the aging process varies from individual to individual and from breed to breed. The smallest breeds in this study do probably not have the same progression of the aging process as the largest breed. In the present study, the majority of the dogs are medium sized- and large breeds (Table 1). Whether the difference in average age of about one year is of importance in this study is uncertain, when differences in the histological material are taken into consideration.

Glomerular changes

Most of the pyometra dogs had normal or just slight degree of glomerular changes (Table 3). Only two dogs revealed moderate degree of glomerular changes. In comparison, the minority of the control dogs were normal, four dogs revealed moderate, and two dogs showed severe degree of glomerular changes (Table 3).

The present study reveals that pyometra dogs do not have glomerular changes worse than the age-matched control dogs. This result is supported by a previous study,[12] that experienced that the prevalence and severity of glomerular changes detected by light microscopy in 27 dogs with pyometra were similar to a group of 16 age-matched control dogs. It was assumed that these changes most likely were age-related.

Another study[9] came to different conclusion concerning renal lesions in pyometra dogs. All 23 pyometra bitches included in the study revealed glomerulonephritis by light microscopy. Much of today's literature is based on this conclusion, although the dogs only were compared to five normal dogs. These dogs were not age-matched to the pyometra bitches. One cannot know from this study, in what degree the age related changes accounted for these lesions. Heiene et al[5] found by light microscopy that some pyometra dogs had serious glomerular changes. Questions raised in the study were whether these changes indicated permanent renal lesions and whether they were related to pyometra. Further research was recommended.

Age-related changes

To avoid misdiagnosing old dogs there is need of knowledge about normal age related renal changes in this species. The changes in renal structure that occur as a result of aging, have been well documented in rodents and people.[2,7] The most characteristic lesion of renal aging

is glomerular sclerosis. In man the changes in renal structure begin in the third or fourth decade of life and accelerate appreciably after the seventh decade. Age-related lesions similar to those observed in aging people, have also been observed in aging dogs in two studies.[3,4] Beyond these, there are few studies done on this subject.

The criteria for the selection of dogs for the control group were female dogs older than seven years without signs of PU/PD or other internal medicine conditions. Still, the kidney in these dogs could have been under the influence of other less severe diseases, not reported by the submitting veterinary at the time of necropsy. Small skin infections and mild otitis externa are such examples. In the light of this, it seems probable that geriatric dogs in general, reveals some degree of renal changes.

In a study on 230 greyhounds submitted to Kansas state university for necropsy,[10] there were found that out of 25 old dogs (22 females) without clinically evident renal failure, 64 % had glomerulonephritis identified by light microscopy.

In the present study, renal glomerular changes were found in the old dogs in the control group, often without clinical signs of renal failure. The histopathology seems to offer little information about the clinical severity or the prognosis.

Interstitial changes

Eight dogs in the pyometra group revealed interstitial cell infiltrations of slight or moderate degree in the cortex. In comparison, only one dog from the control group had interstitial cell infiltration (Table 2). The interstitial nephritis in the pyometra dogs seemed to be sub acute or acute in nature, since no signs of sclerosis were noted. The only exception was dog P16 that revealed both chronic interstitial nephritis and glomerulonephritis. This dog had clinically evident renal disease at the time of surgery. The interstitial nephritis also seemed to occur

independently of glomerulonephritis (See Table 2). This was also the conclusion in a previous study [8], which evaluated the interdependence of glomerular and interstitial changes in 101 dogs with spontaneous glomerulonephritis.

Histological techniques

The accuracy of the study may be discussed, because detection of renal changes by light-microscopy depends largely on adequate histological techniques. In the present study the staining used was H&E and van Gieson. The slightest degree of glomerular changes tended sometimes to be overlooked without special staining. Especially the basement membrane was hard to evaluate. In comparison interstitial cell infiltrations was detected easily without further staining techniques. The next step in this study would be to investigate all the specimens with special staining techniques like PAS, Silver methenamine, and Jones'periodic acid. Electron microscopy and immunhistochemistry would also be of advantage, and if the evaluations were carried out double-blinded, misdiagnosing would further be avoided.

In human medicine morphometri is used as an effective tool in morphological investigations of the kidney. This tool could be used in veterinary medicine as well, to describe the canine renal morphology in a more precise and objective way.

Questionnaire

To express an opinion whether pyometra results in chronic renal failure, a questionnaire was made to owners of dogs that joined in a previous study on pyometra dogs.[5] The owners' answers about their dogs, revealed that out of 41 dogs just 5 had been drinking more than usual after the surgery or towards the end of their lives and only 2 dogs died of renal failure. The two latter ones had a severe proteinuria after the surgery as well. Proteinuria was in a

large human study[6] found as a strong independent predictor on end stage renal disease in a setting of mass screening. Even a slight increase in proteinuria was an independent risk factor for end stage renal disease (ESRD). It is reasonable to believe that this accounts for dogs as well. This study supports the results in the questionnaire. The validity of the results from the questionnaire is uncertain, because of the unknown prevalence in the geriatric dog population. The accuracy of the questionnaire may be discussed because of the long time passing. Clinical signs of renal failure might be difficult to remember for the owners approximately 8 years after the surgery.

CONCLUSION

To conclude, glomerular damage beyond age-related changes could not be demonstrated by light microscopy in these dogs with pyometra. Age-related renal changes in the dog include glomerular lesions. Proteinuria may persist in patients after ovariehysterectomy and predispose for the development of renal failure.

LITERATURE

1. Bloom F. The extragenital lesions of canine pyometra. *Cornell Veterinarian* 1946; 36: 324-342.
2. Brown WW, Davis BB, Spry LA, et al. Aging and the kidney. *Arch Intern Med* 1986; 146: 1790-1796.
3. Cowgill LD, Spangler WL. Renal insufficiency in geriatric dogs. *Vet Clin North Am Small Anim Pract* 1981; 11: 727-748.
4. Guttman PH, Andersen AC. Progressive intercapillary glomerulosclerosis in aging and irradiated beagles. *Radiat Res* 1968; 35: 45-60.
5. Heiene R, Moe L, Molmen G. Calculation of urinary enzyme excretion, with renal structure and function in dogs with pyometra. *Res Vet Sci* 2001; 70: 129-137.
6. Iseki K, Ikemiya Y, Iseki C, et al. Proteinuria and the risk of developing end-stage renal disease. *Kidney Int* 2003; 63: 1468-1474.
7. Lindeman RD. Changes in renal function with aging. Implications for treatment. *Drugs Aging* 1992; 2: 423-431.
8. Muller-Peddinghaus R, Trautwein G. Spontaneous glomerulonephritis in dogs. II. Correlation of glomerulonephritis with age, chronic interstitial nephritis and extrarenal lesions. *Vet Pathol* 1977; 14: 121-127.
9. Obel A-L, Nicander L, Åsheim Å. Light and electron microscopic studies of the renal lesion in dogs with pyometra. *Acta Veterinaria Scandinavica* 1964; 5: 93-125.
10. Schoning P, Cowan LA. Gross and microscopic lesions of 230 Kansas greyhounds. *J Vet Diagn Invest* 1993; 5: 392-397.
11. Stone EA, Littman MP, Robertson JL, et al. Renal dysfunction in dogs with pyometra. *J Am Vet Med Assoc* 1988; 193: 457-464.
12. Stone EA, Littman MP, Robertson JL, et al. Renal dysfunction in dogs with pyometra. *Journal of the American Veterinary Medical Association* 1988; 193: 457-464.

SAMMENDRAG

NYRESKADER HOS HUNDER MED PYOMETRA SAMMENLIGNET MED EN ALDERSMATCHET KONTROLLGRUPPE: LYSMIKROSKOPISK UNDERSØKELSE AV INTERSTITIELLE OG GLOMERULÆRE FORANDRINGER , OG UTVIKLING AV KLINISK NYRESVIKT

Hvorvidt hunder med pyometra utviser forandringer av kronisk karakter i nyrene er et kontroversielt tema. Målet med denne studien er å undersøke om pyometra hunder har nyreskader utover de forandringer denne aldersgruppen normalt ville ha pådratt seg gjennom et langt liv uten pyometra. Om risikoen for å utvikle permanent nyresykdom er større for pyometra hundene ble også undersøkt.

Nyre biopsier fra 19 hunder med pyometra fra et tidligere studium, ble undersøkt og klassifisert ved lysmikroskopi, og sammenlignet med 16 aldersmatchede kontroll hunder som ble undersøkt på samme måte. Kontrollhundene var enten døde eller var blitt avlivet av årsaker uten kjente indremedisinske komponenter. Det var de glomerulære og interstitielle forandringene som ble fokusert på i sammenligningen av de to gruppene.

41 pyometra hunder som hadde gjennomgått ovariehysterektomi ble fulgt opp i et telefon intervju med eierne ca 8 år senere. Målet var å finne ut hvor mange av hundene som var døde av, eller som hadde hatt symptomer på nyresvikt etter operasjonen.

Den histopatologiske undersøkelsen viste at de glomerulære forandringene (hovedsakelig av glomerulonefritisk og glomerulosklerotisk karakter) var mer uttalt hos kontrollhundene sammenlignet med pyometra hundene. Uttalt grad av interstitiell mononukleær celleinfiltrasjon ble bare funnet hos pyometra hundene.

Av de 41 hundene som deltok i den retrospektive spørreundersøkelsen, hadde fem hunder drukket mer enn vanlig etter operasjonen og fortsatt med dette resten av livet. 24 hunder var døde eller de var blitt avlivet av tilstander som tilsynelatende ikke var relatert til nyresvikt. Dødsårsaken var imidlertid av mer usikker årsak hos 11 hunder. To hunder ble avlivet med nyresvikt som dødsårsak. Disse hundene hadde også uttalt proteinuri ved operasjons tidspunktet i den opprinnelige studien. Fire hunder levde fremdeles under telefon intervjuet.

Som en konklusjon, kunne glomerulær skade utover de aldersrelaterte, ikke demonstreres ved lysmikroskopi hos pyometra hundene i denne studien. Aldersrelaterte forandringer ser ut til å inkludere en viss grad av glomerulære forandringer. Proteinuri kan persistere etter ovariehysterektomi og predisponere for utvikling av nyresvikt.

APPENDIX 1: Questionnaire

Reidun Heiene, dr.scient., 1. amanuensis
Institutt for smådyrsjukdommer
Norges veterinærhøgskole
P. b. 8146 dep.
0033 OSLO
tlf.:22 96 45 00

Til eier av hund som avlives

Spørsmål om nyrer

Vi har forstått at din hund skal avlives og er klar over at dette er et svært vondt øyeblikk for deg/dere. Vi føler med dere i den situasjonen.

Da det kan være til nytte for andre hunder, spør vi likevel om å få ta en liten vevsprøve fra et nyre gjennom huden etter at hunden er død. Det endrer ikke noe av det som ellers skjer med hunden ved avliving eller etterpå.

Dette er en del av en undersøkelse hos hunder som dør av andre årsaker enn nyresvikt. Målet er å få hjelpemidler til å finne faresignaler for nyresykdom. Det vil også kunne hjelpe pasienter med nyreskader. For å tolke vevsprøver må man vite hva som er normale forandringer etter et langt liv.

Det må derfor være kjent om hunden har hatt tegn på nyresykdom eller andre sykdommer.

Har hunden drukket mer og tisset mer enn vanlig i det siste? Ja Nei (kryss av)

Har hunden hatt urinveisinfeksjon i det siste? Ja Nei (kryss av)

Har hunden hatt vekttap eller nedsatt allmenntilstand i det siste? Ja Nei (kryss av)

Har hunden hatt andre langvarige eller alvorlige sykdommer tidligere? Ja Nei I så fall hva slags sykdom?_____

Eiers underskrift_____

Hvis du fyller ut skjemaet, takker vi for det, og vil gjøre vårt beste for at resultatene skal komme framtidige pasienter til gode. Ved spørsmål kan undertegnede kontaktes på Institutt for smådyrsykdommer, Norges Veterinærhøgskole (22 96 45 00)

Vennlig hilsen

Reidun Heiene (ansvarlig veterinær)

Appendix 2: Protokoll for evaluering av nyrehistologi:

Journalnummer:
Glomerulære forandringer:
Cellerikdom:
Basalmembranforandringer:
Kapillærenes lumenstørrelse:
Homogenitet (drag,områder,avleiringer):
➢ Kollagen reaksjon(VG-snitt):
➢ Fibrocyttkjerner:
➢ Amyloid:
Bowmans kapsel(fortykket,dillatert,crescentdannelse o.a.):
Uric space:
Interstitielle forandringer:
Hyalinitet (drag, områder):
Celleinfiltrasjon:
Diagnose:

Journalnummer:
Glomerulære forandringer:
Cellerikdom:
Basalmembranforandringer:
Kapillærenes lumenstørrelse:
Homogenitet (drag,områder,avleiringer):
➢ Kollagen reaksjon(VG-snitt):
➢ Fibrocyttkjerner:
➢ Amyloid:
Bowmans kapsel(fortykket,dillatert,crescentdannelse o.a.):
Uric space:
Interstitielle forandringer:
Hyalinitet (drag, områder):
Celleinfiltrasjon:
Diagnose:

Journalnummer:
Glomerulære forandringer:
Cellerikdom:
Basalmembranforandringer:
Kapillærenes lumenstørrelse:
Homogenitet (drag,områder,avleiringer):
➢ Kollagen reaksjon(VG-snitt):
➢ Fibrocyttkjerner:
➢ Amyloid:
Bowmans kapsel(fortykket,dillatert,crescentdannelse o.a.):
Uric space:
Interstitielle forandringer:
Hyalinitet (drag, områder):
Celleinfiltrasjon:
Diagnose:

APPENDIX 3: PICTURES OF RENAL SECTIONS FROM THE HISTOLOGICAL INVESTIGATION

Classification of renal changes in pyometra- and control dogs:

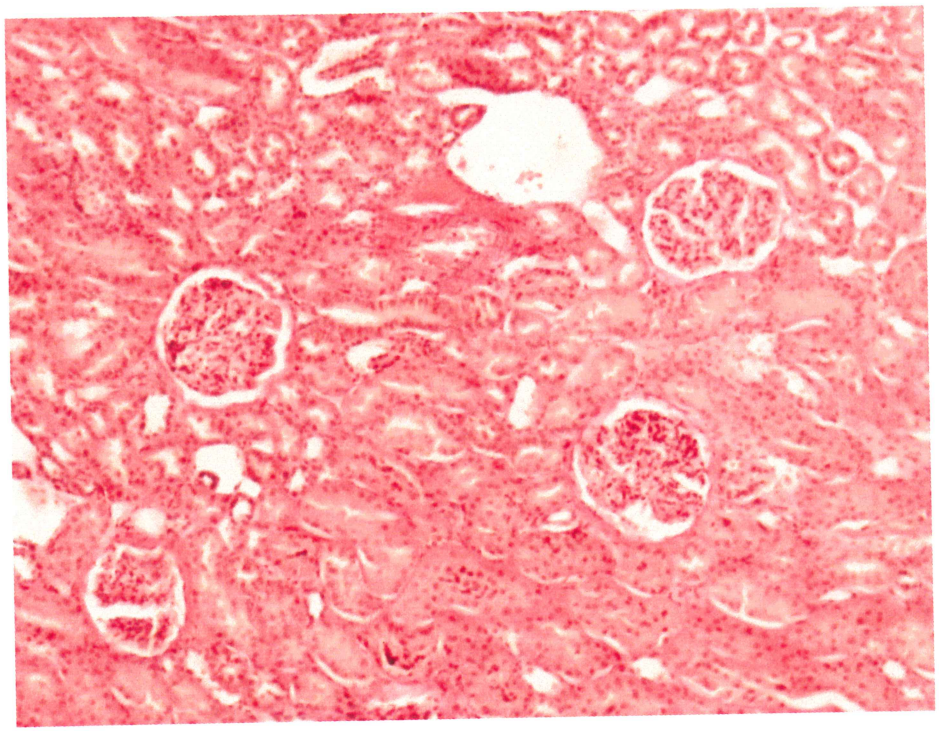


Fig.3: Normal canine kidney
H&E-stain, x10 (Dog C15)

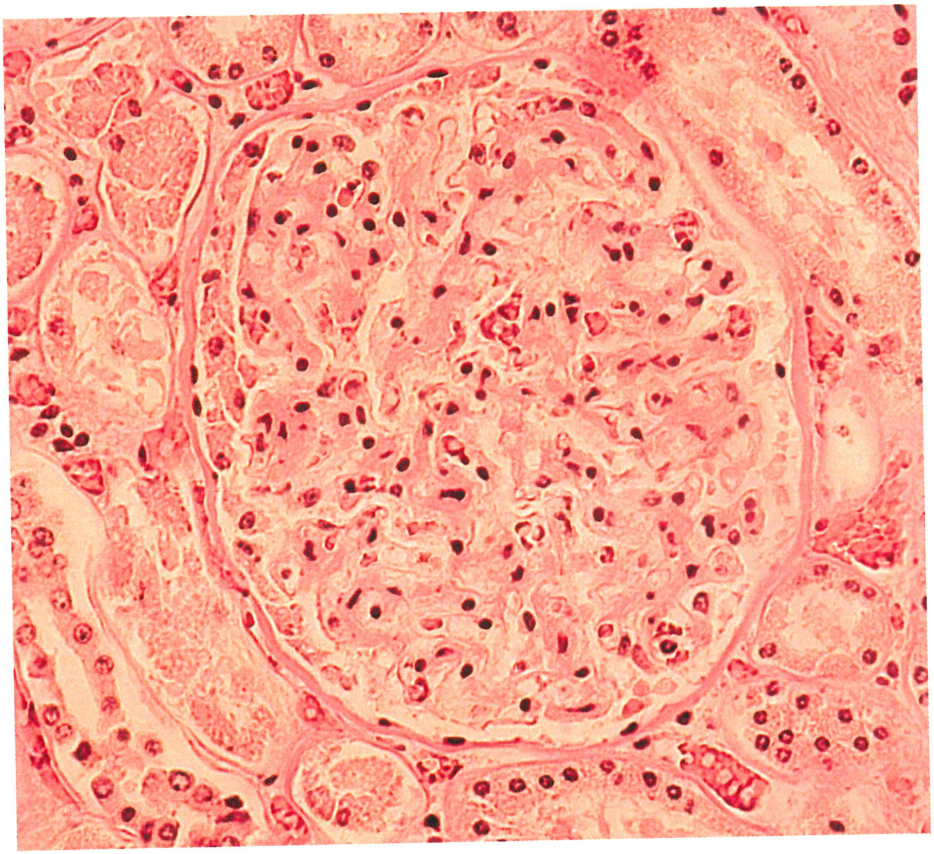


Fig 4: Slight degree of glomerular changes
Thin strains of sclerosis is visible through-out the glomerulus as homogenous red coloured regions. A few spindle-shaped cells are dissiminated in the glomerulus.
Van-Gieson-stain, x40, (Dog C13)

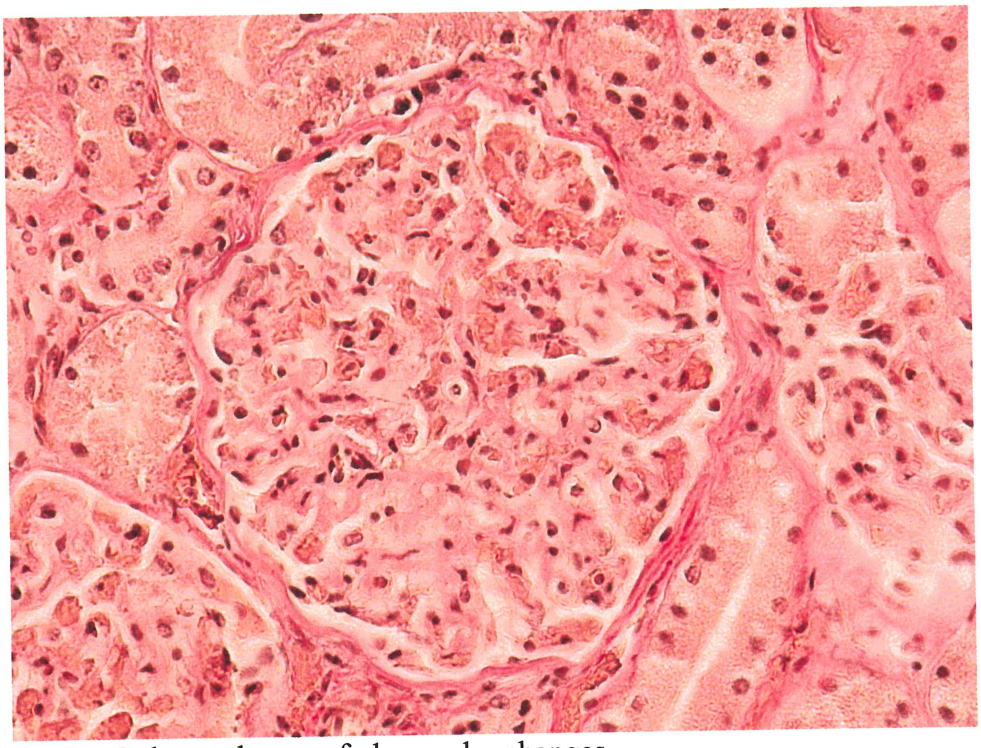


Fig.5: Moderate degree of glomerular changes
The glomerulus reveals large regions of homogenous appearance. Several spindle –shaped cells is also scattered in the glomerulus. The picture gives the impression of having thickened basement membranes, but this is not verified by special staining
Van-Gieson-stain, 40x, (Dog C9)

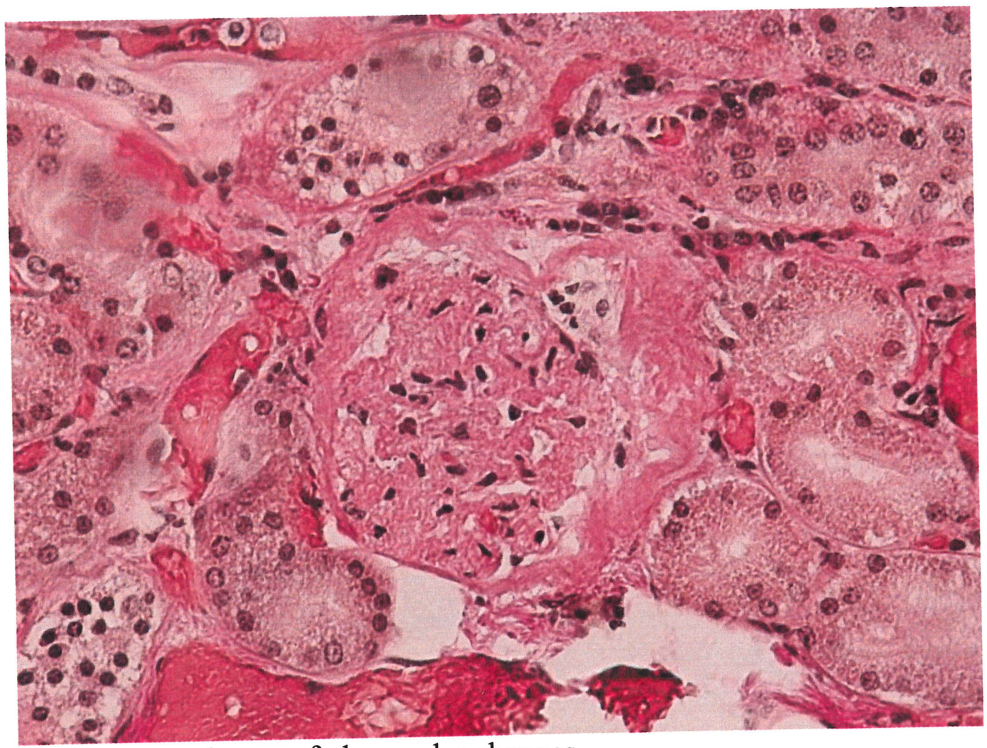


Fig.6: Severe degree of glomerular changes
A dog in this category would reveal many glomeruli with almost global sclerosis. The majority of the cells in the glomeruli are spindle-shaped.
Van-Gieson stain, 40x, (dogC7)

A B

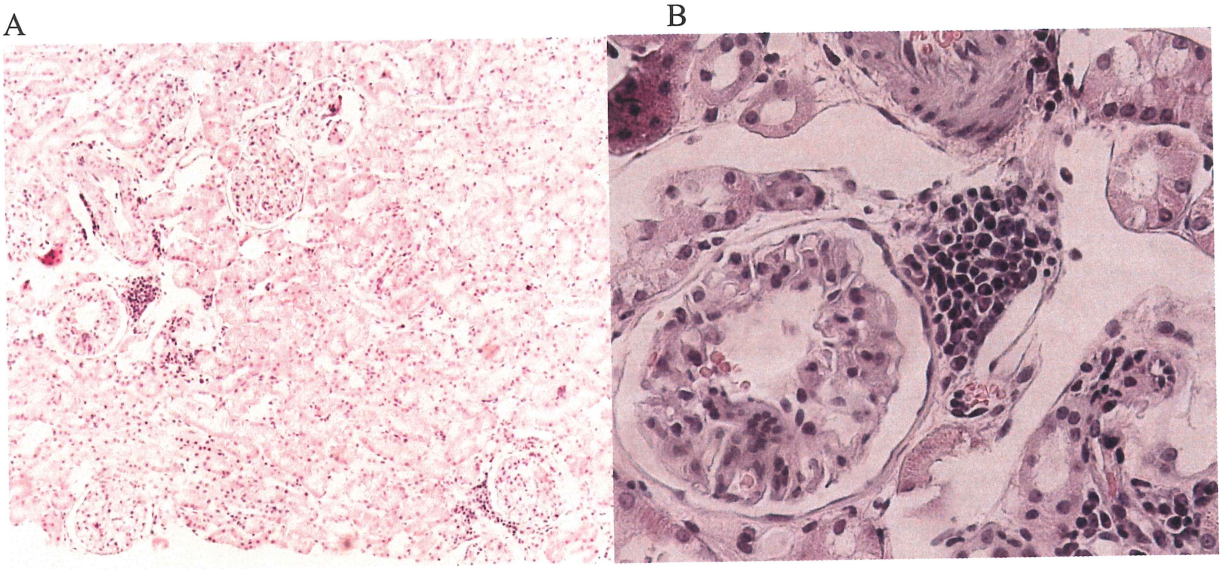


Fig.7: Slight degree of interstitial changes

Slight periglomerular infiltrates of lymphocytes and plasmacells were observed in the majority of the pyometra dogs, but seldom in the control dogs.

7A: H&E-stain, x10,(Dog P11)

7B: H&E-stain, x40,(Dog P11)

A B

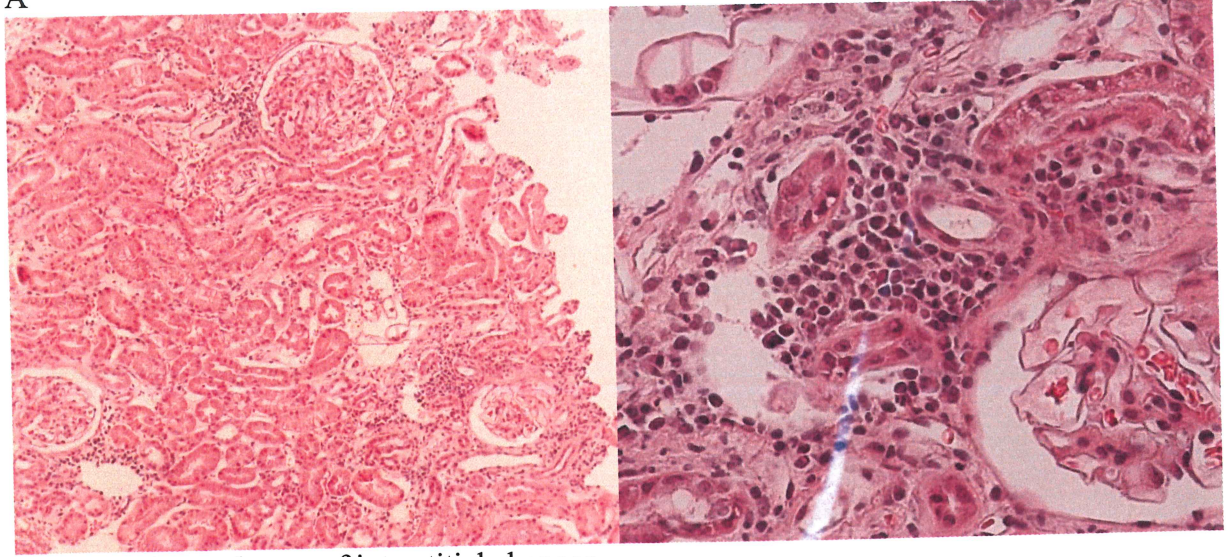


Fig.8: moderate degree of interstitial changes

A large amount of mononucleated cells was observed in this category. A periglomerular distribution of the inflammation cells was typical in the pyometra dogs.The mononuclear cells basically consisted of plasma cells and lymphocytes(8B).

8A:H&E-stain, x10, (Dog P8)

8B: H&E-stain, x40, (Dog P8)

Other changes observed , but not categorized:

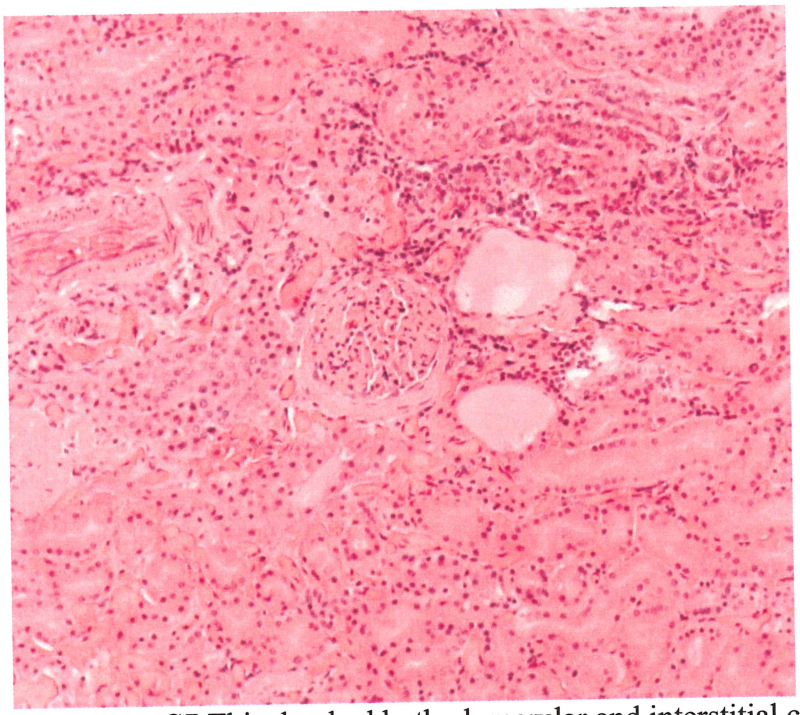


Fig.8: Dog C7.This dog had both glomerular and interstitial changes of moderate degree. Here a thickened Bowman's capsule also is revealed, together with moderate degree of cell infiltration.
H&E-stain, x10 (Dog C7)

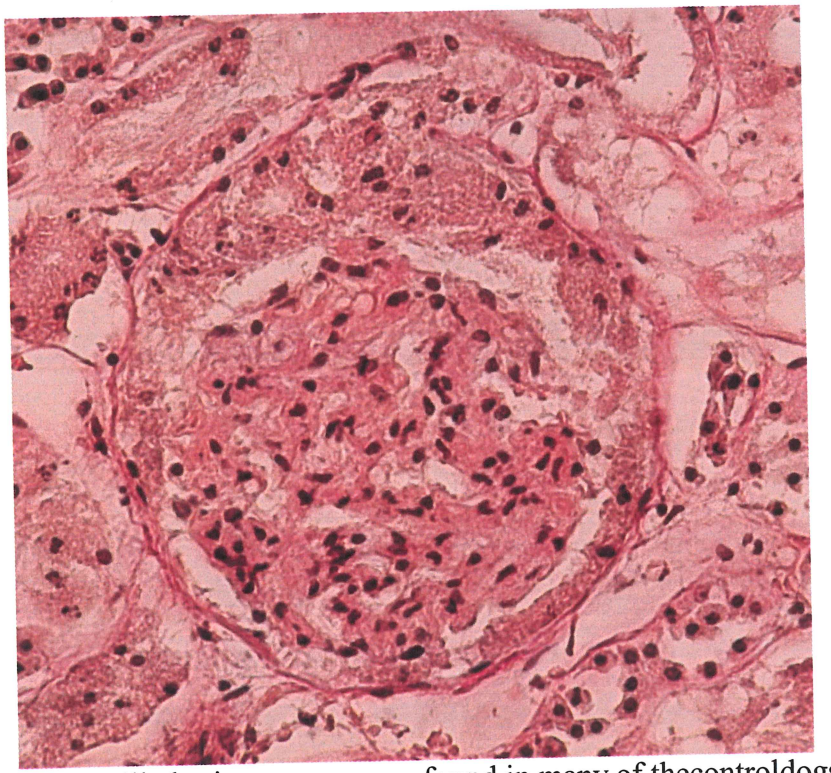


Fig.9: Filled urinary space was found in many of thecontroldogs, probably because of backflow from tubuli in the post mortem period.
Van Gieson-stain, x40