DIAGNOSTIC STUDY AND OBSERVATIONS OF THE UROGENITAL SYSTEM OF MALE DOGS INFECTED WITH BRUCELLA CANIS

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ABSTRACT

DIAGNOSTIC STUDY AND OBSERVATIONS
OF THE UROGENITAL SYSTEM OF MALE DOGS
INFECTED WITH BRUCELLA CANIS

by John A. Moore

Seventeen male dogs, naturally infected with Brucella canis, were utilized in an effort to obtain findings specific for the disease. Disease pathogenesis and its effects on the urogenital system were closely observed. Presence of a bacteremia, agglutinating serum antibodies and pathologic lesions of the urogenital organs were studied in greater depth than were hemograms, urinalyses, and blood chemistries.

All infected animals possessed a bacteremia which was transient in some animals. Agglutinating serum antibodies were also detected in all infected animals and in a portion of the normal dogs. The significance of this finding in normal dogs is not known.

Epididymitis and/or testicular atrophy was sometimes seen either bilaterally or unilaterally in diseased dogs.

Hematologic, urologic and blood chemistry studies yielded inconsistent results. A Brucella canis bacteriuria was found in samples collected by bladder puncture in eight of twelve dogs.

The infection produced a reticuloendothelial response which was most pronounced, and always observed in the prostate gland. Lesions were also consistently noted in the kidney, ductus deferens, and

epididymis. An aspermia, seminiferous tubular degeneration and testicular fibrosis were seen in several adult animals.

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By

John A. Moore

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INTRODUCTION

A disease of the dog caused by the bacterium Brucella canis was first reported in October 1966. Initial reports primarily discussed findings in diseased bitches and their offspring.

Aside from the observation that infected male dogs had been detected, essentially no study of this sex had been undertaken.

The author was cognizant of the fact that male dogs usually have venereal access to significant numbers of females and as a result could command a major role in dissemination of the disease.

Observations and study of infected male dogs commenced with special effort expended towards finding diagnostic means for detecting diseased animals and in defining the pathologic effects on the urogenital system.

LITERATURE REVIEW

Reports of disease in the dog caused by bacteria in the genus Brucella are few in number.

Brucella abortus infection in a female dog apparently caused abortion during the 54th day of pregnancy according to Morse et al. (1953).

A male dog with a unilateral orchitis and pyrexia was observed for six weeks with no remission of symptoms. Surgical removal of the enlarged testis was effected. Brucella suis was isolated by Planz and Huddleson (1931) from a large abscess present in the specimen. A serum sample obtained from the dog contained agglutinating antibody for the bacterial isolate at a dilution of 1:500. A later serum sample showed the agglutination titer persisting at the initial level.

Love et al. (1952) revealed the finding of an epididymitis in a two-year-old male dog in which Brucella abortus was isolated. A brucella agglutination titer at a 1:200 dilution was found in a serum sample procured from the animal. They also observed an elevated temperature (104° F.), listlessness and inappetence in the animal. Abscessation of the surgically removed epididymis was found. The testis was not noted to be involved. Histologic examination found the primary response in the tubule lumens with polymorphonuclear leukocytes the predominant cell type. Some epithelial cells were also noticed. In some areas the duct wall was eroded and the lesion

extended into the interstitial tissue. The vas deferens was not involved in the area of abscessation but infiltration of its wall by plasma cells and a few macrophages was observed.

A ranula between the rami of the mandible in a dog was felt to be due to Brucella sp. on the basis of a brucella agglutination titer of 1:50 in a report from Karlson (1940). Repeated blood culturing over a three-month period failed to isolate a bacterial organism. Another case of brucellosis in a dog was described by Davis (1940). The male dog he observed had a temperature of 105° F. and its left testicle was enlarged. This animal possessed an agglutination titer of 1:2000. After unilateral orchidectomy, an abscess measuring 2.5 cm. x 3.75 cm. was seen in the testis. Davis attempted no direct bacterial isolations but did inoculate some of the abscessed material into guinea pigs. The guinea pigs developed a brucella agglutination titer of 1:25.

In most of the reported cases of brucellosis in the dog, the animal lived in a farm habitat and was often in contact with farm animals known to have brucellosis (Morse et al., 1953; Love et al., 1952; Karlson, 1940; and Delez, 1937).

Feldman (1937) collected serums from 500 dogs in the Minnesota area and found 6.6% to possess brucella agglutinins at a 1:25 dilution or higher. He experienced no success in attempts to isolate Brucella sp. from 14 dogs with a titer. A lower percentage of dogs isolated for at least one month had brucella agglutinin titers than did those monitored upon initial receipt. He concluded that Brucella sp. infections in dogs are of minor significance and little correlation was found between presence of serum agglutinins and actual infection.

Experimental attempts to infect 11 dogs with Brucella suis or Brucella bovis by administration of the organism either intravenously or orally were conducted by Feldman et al. (1935). None of the animals that consumed the organisms became infected. Two animals inoculated intravenously harbored the organisms at necropsy. In one of these latter dogs lesions were observed in liver and lung. Observed lesions were focal and not encapsulated. The primary inflammatory cell seen in the lesions was monocytes; a few lymphocytes were also observed.

Attempts to infect 19 young dogs through the daily feeding of milk, known to contain Brucella abortus, for up to 4 months were unsuccessful in a report published by Morse et al. (1951). All animals also failed to develop brucella agglutinating antibodies. In another study Morse et al. (1951a) attempted to infect dogs with Brucella abortus by feeding 15 adult animals aborted bovine fetuses and placentas known to be infected with the organism. They concluded that dogs may harbor Brucella abortus in one or more lymph nodes following a single infected meal. The presence or absence of brucella agglutination titers had no bearing on whether dogs harbored the organism. They also found a dog that developed a Brucella sp. bacteremia.

Kerby et al. (1943) studied the effects of repeated intravenous or intraperitoneal injections of Brucella suis in 9 dogs. All animals developed agglutination titers. Those inoculated intravenously eliminated the organism from their blood stream, usually within three weeks. Bacteriologic isolation from tissue was successful in some instances 3 to 7 months later. The intraperitoneal route of inoculation gave poor results. Margolis et al. (1945) reported the histopathologic

lesions seen in Kerby's (1943) experiment. The significant finding was a sinusoid reaction within the lymph nodes in which large masses of mononuclear cells accompanied by polymorphonuclear cells were seen.

Carmichael (1966), in a news report, commented on the isolation of a Gram negative coccobacillus from bitches that had aborted. Serologic and bacteriologic studies of various specimens confirmed similar abortion episodes in 13 states.

Moore and Bennett (1967) reported the characteristics of an organism which they had recovered from specimens procured from aborting bitches and their expelled fetuses. The organism appeared to be identical to the one recovered by Carmichael. On the basis of preliminary studies they suggested the organism be named Brucella canis.

Taul, Powell and Baker (1967) reported the recovery of an unclassified Gram negative bacterium from animals originating from four different South Carolina kennels. They found the coccobacillus to be biochemically and antigenically related to both the genera Brucella and Bordetella. Infertility and abortion in females, and orchitis in males, seemed to be the predominant signs of infection. The authors further stated that the disease is spread by animal contact and that infection can be induced via the oral route.

Moore (1967) presented his observations on the epidemiologic, serologic, bacteriologic and histopathologic features of the spontaneous disease. His attempts at treatment of the disease were unsuccessful. Early results on control and eradication procedures, predicated on a test and slaughter program, appeared to have been effective.

Carmichael (1967) recently presented a report, "Canine Brucellosis: Isolation, Diagnosis and Transmission". He was able to transmit the

infection by intravenous, subcutaneous, oral, conjunctival, and intravaginal routes as well as by contact exposure. The time betweeen oral inoculation and the onset of a bacteremia was 3 weeks. Dogs inoculated intravaginally all became bacteremic within one week. Susceptible dogs placed in contact with a bitch that had aborted two days previously became bacteremic one to three weeks later. It was suggested that virtually all mucous membrane surfaces are susceptible to the canine Brucella in a manner similar to brucella infections in other species. Serologic studies found that antiserum to other Brucella sp. does not react strongly if at all with the canine organism, although serums prepared against rough brucellae and Brucella ovis will agglutinate Brucella canis. Significant agglutination titers to Brucella canis were found in 2 of 24 Texas cottontail rabbits and 8 of 20 Canadian goose serums. Whether these represented heterologous reactions was not defined.

Hill, Van Hoosier and Wende (1967) also related their observations of a Beagle production colony in which abortions were found. A Gram negative coccobacillary organism was incriminated as the etiologic agent. Their observations paralleled those of Carmichael (1967) and Moore (1967).

MATERIALS AND METHODS

ANIMALS

Twenty-seven male Beagle dogs were used in the study; 10 were normal and 17 infected with Brucella canis.

The normal animals were adults housed in a rural kennel and actively utilized for breeding during the period they were monitored.

Ten of the 17 infected male dogs were adults. They had been previously removed from the same rural kennel when found to possess a Brucella canis bacteremia. Upon removal from the kennel they were transferred to the Veterinary Clinic Building, Michigan State University.

Three other infected male litter mates were received at 94 days of age suffering a minor respiratory disturbance. Antibiotic therapy for several days resulted in an immediate return to good health. Brucella canis had been isolated from a blood culture and vaginal swab collected from their dam. A litter mate had died in the first few days of life and Brucella canis was isolated from its tissues.

Four other litter mate males were obtained at birth. The litter had been cesarean derived utilizing gnotobiotic technique and rearing practices. Bacteriologic swabs taken from amniotic fluid at time of surgery yielded Brucella canis in pure culture. The animals remained in an isolator for 34 days and were then transferred to cages in the Veterinary Clinic.

All infected animals (except litter mates) were separately housed and given access to food and water at all times. Purina Laboratory Chow was the feed source, and the "Principles of Laboratory Animal Care"* were strictly adhered to throughout the course of study.

All infected animals were observed daily for any overt change in behavior and were periodically examined for any physical abnormality.

BACTERIOLOGIC CULTURES

Blood

Culture bottles were prepared by slanting 18 ml of Tryptose Agar (Difco) along one side of a 100 ml screw capped milk dilution bottle. Immediately preceding use, 15 ml of Brain Heart Infusion Broth (Difco) were added to each bottle. Two cc of blood were collected aseptically from the dog's external jugular vein and immediately placed in the prepared culture bottle.

All cultures were continuously incubated at 37° C. Cultures were examined for bacterial growth every 48-72 hours until 21 days of incubation had elapsed. Samples which failed to elicit bacterial growth at the end of this period were discarded.

Culture bottles evidencing bacterial growth were removed from the incubator and an aliquot from the bottle streaked on a Tryptose Agar (Difco) plate enriched with 6.5% ox blood. After 96 hours' incubation, an isolated colony was characterized by inoculation into various differential media as described by Moore and Bennett (1967).

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* American Association for Laboratory Animal Science, Joliet, Illinois.

Six infected male dogs were monitored for the persistence of a bacteremia (Appendix 1). The monitoring period extended from 3 to 5 months. A total of 25 blood culture samples was obtained. Each dog was tested at least 2 times during this period.

Ten normal adult male dogs were monitored for the presence of a bacteremia over a period of 4 to 6 months (Appendix 1). A total of 35 blood culture samples was obtained with no less than 3 from each dog.

Tissues

Concerted attempts were made to secure all tissues in a sterile manner. After collection, each tissue was seared in an open flame, excised with sterile scissors exposing a fresh surface which was impressed lightly on a Tryptose Agar plate enriched with 6.5% ox blood.

After streaking, the plates were incubated at 37° C. and observed daily for growth. Isolated colonies were further characterized in a manner identical to those described by Moore and Bennett (1967).

SEROLOGY

Serum

The same 6 infected male dogs monitored for the presence of a bacteremia were also tested during the same period for agglutinating serum antibody (Appendix 1). Twenty-two serum samples were collected during this period with each dog represented by at least 3 samples.

The 10 normal dogs monitored bacteriologically were also simultaneously monitored for the presence of agglutinating antibody (Appendix

1). A total of 41 samples was obtained with each dog represented by at least 3 samples.

Blood was collected from the external jugular vein, immediately transferred from the syringe into a clean glass test tube, and allowed to clot at room temperature. After a firm clot had formed, the sample was refrigerated at 5° C. for at least one hour. The clot was then loosened from the test tube wall and the entire sample centrifuged at 2000 r.p.m. (Model CS International Centrifuge) for 30 minutes. The serum was pipetted into a clean container which was then sealed and frozen until needed.

Antigen Preparation

A pure culture of Brucella canis was inoculated into Brain Heart Infusion Broth (Difco) and incubated for 18 hours at 37° C. This 18 hour broth culture was used to seed a 16 oz. prescription bottle containing 100 ml Tryptose Agar (Difco) slanted on one side. The seeded prescription bottle was incubated at 37° C. for 48 hours. The confluent bacterial growth was removed from the agar slant using sterile glass beads and phosphate buffered saline, pH 7.2 (PBS). The liquid suspension of the organism was decanted into a sterile erlenmeyer flask, and 0.05% buffered formalin, pH 7.2, added. The formalized suspension was incubated at 37° C. for 4 hours, then centrifuged at 2500 r.p.m. in a Model CS International Centrifuge for 30 minutes. The supernatant was decanted and discarded. Phosphate buffered saline was added to the tube and the sediment resuspended. Similar centrifugation, decanting, and resuspending procedures were done until the organisms were washed 3 times. After final washing the sedimented

cells were again resuspended in a small amount of phosphate buffered saline and heat treated in a 56° C. water bath for 30 minutes. The preparation was then stored at 5° C. as a "stock" preparation. The final antigen preparation was prepared each day just prior to use. The required volume of the "stock" suspension was diluted in the appropriate amount of PBS, until a reading of 22% light transmission at 420 mμ was obtained with a spectrophotometer.

Tube Agglutination Test

Candidate serums were thawed and dilutions prepared in 10 x 100 mm glass test tubes using PBS as the diluent. An initial 1:12.5 dilution was made and subsequent twofold dilutions prepared until a dilution of 1:400 was obtained in the sixth tube. Each serum tube dilution contained 0.5 ml. The prepared antigen was then added in 0.5 ml amounts to each serum tube. Thus each serum had final dilutions of 1:25, 1:50, 1:100, 1:200, 1:400, and 1:800. An undiluted antigen control, as well as a serum known to possess an agglutination titer of 800 and another serum previously shown to possess no agglutinating properties, were prepared each time.

The tubes were incubated at 37° C. for 20 to 24 hours. Each tube was read in a darkened room under a single source of direct light. After reading, all tubes were classified on a numerical scale of 0 to 4 as follows:

0 No agglutination or settling of bacterial cells.

1 Slight random settling of bacterial cells noted on the tube bottom but essentially all cells in homogeneous suspension.

2 Moderate settling of bacterial cells, a large proportion of cell still in homogeneous suspension. Some margination of settled cells. No clumping noted.

3 Moderate to marked settling of bacterial cells. Some clumping of cells noted. Some cells still in suspension.

4 Total settling of all cells leaving a clear supernatant. Clumping of cells noted.

The tube of highest dilution possessing a 3 classification was deemed the end point. Titer was expressed as the reciprocal of the dilution possessing the end point.

HEMOGRAMS

Ten infected dogs were selected for periodic hematologic studies prior to euthanasia (Appendix 2).

Blood samples for hemoglobin, packed cell volume determination, total erythrocytes, total leukocyte, and differential leukocyte counts were collected in vacuum tubes containing potassium ethylenediaminetetraacetate (EDTA) as the anticoagulant.

Hemoglobin values were determined by the cyanmethemoglobin method and read at 540 mμ wave length using a Fisher Flowthrough Photometer. The packed cell volumes were determined by the capillary tube method.

Smears for differential leukocyte counts were made on coverslips, dried in air and stained with Wright's stain.

Erythrocytes and leukocytes were counted, using an electronic cell counter (Coulter) adjusted to thresholds 11 and 20, respectively, with an aperture current setting of 0.707 for each.

BLOOD CHEMISTRY

Fresh serums were used for blood urea nitrogen (BUN) and creatinine determinations using the Conway Microdiffusion method (1958)* and the method of Folin and Wu (1954), respectively. These determinations were made on 8 infected males (Appendix 2).

URINALYSES

Eleven infected dogs were utilized for urinalyses (Appendix 2). Urine was secured by catheterization and examined for occult blood, protein, pH, glucose, and urine bilirubin using the Ames Clinitest** reagents. Aliquots of the urine specimens were centrifuged at 1000 r.p.m. in a Model CS International Centrifuge for 5 minutes. The supernatant was decanted and a drop of Sedi-Stain (Clay Adams)*** was mixed with the sediment. A drop of the mixture was transferred to a clean glass slide and a coverslip applied. The prepared slide was examined microscopically with reduced light.

NECROPSY

Ten animals were euthanatized using intravenous sodium pentobarbital and necropsy examinations were performed (Appendix 2).

At necropsy, samples of bladder urine, prostate gland, testicle, spleen, liver, and kidney were usually collected for bacterial isolation attempts.

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* Urograph, Warner Chilcott, Morris Plains, N.J.

** Ames Company, Elkhart, Indiana

*** Clay-Adams, Incorporated, New York, N.Y.

Samples of testicle, epididymis, vas deferens, prostate gland, bladder, ureter and kidney were collected and placed in either Bouin's fixative or 10% neutral buffered formalin for fixation. Tissues were subsequently paraffin embedded, cut at 6 μ thickness and stained with hematoxylin and eosin.

FINDINGS

ANIMALS

No physical abnormalities were noticed in the infected dogs upon initial examination performed within a few days of receipt.

Three infected animals (23032, 23035, 23075) reexamined approximately 4 months after receipt into the Veterinary Clinic were found to have testicular abnormalities.

23032 - Slight bilateral atrophy of the testicles.

23035 - Moderate atrophy of the right testicle and marked enlargement of the left epididymis.

23075 - Marked atrophy of the right testicle and moderate enlargement of the right epididymis.

SEROLOGY AND BLOOD CULTURE

None of the blood samples obtained from the normal dogs yielded Brucella canis.

Six of the 10 normal animals had no agglutination antibodies against the Brucella canis antigen. Four dogs had an initial titer of 200 which decreased to 100 by the end of the observation period. The 6 infected dogs possessed a Brucella canis bacteremia at the beginning of the monitoring period. Four of the animals (23032, 23035, 23068, 23075) failed to yield Brucella canis on blood culture at the end of the monitoring period. One of the four, 23068, was subsequently euthanatized and the organism was isolated from prostate gland.

The six infected dogs had an initial Brucella canis agglutination titer of 800. This titer persisted in 2 of the dogs throughout the monitoring period. Of the 4 whose titers failed to persist:

One (23075) had a final agglutination titer of 400.

Two (23032, 23068) had final agglutination titers of 200.

One (23035) had a final agglutination titer of 100.

The results of bacteriologic culturing of tissues and results of serologic testing of serum obtained at time of necropsy is compiled in Table 1.

HEMATOLOGY

Four of the 10 infected dogs were observed hematologically from birth (30223, 30224, 30225, 30226). Examinations revealed a progressive lowering of hemoglobin, packed cell volume and erythrocytic count during the early weeks of life. The lowest values were observed during the fourth to fifth week; subsequent samples noted a steady increase and plateauing of these values once within the accepted normal range.

This same group demonstrated an increased leukocytic count during the first few days of life. In each instance the leukocytosis resulted from an absolute neutrophilia. The values returned to normal range between the 6th and 14th day. Two dogs (30225, 30226) when sampled at almost 3 months of age evidenced a leukocytosis by virtue of an absolute increase of lymphocytes. This lymphocytosis persisted until they were euthanatized at 167 days of age.

The other 6 infected dogs (23068, 23098, 23037, 20155A, 20155B, 20155C) were sporadically monitored once known to be diseased. Hemoglobin, packed cell volume and erythrocytic counts were consistently within normal range and showed little fluctuation.

Dogs 20155A and 20155B did have an elevated leukocytic count when received at 94 days of age. In both instances an absolute neutrophilia was seen.

Two dogs (20155A, 23037) showed a marked increase in the total leukocytic count at necropsy. In each instance an absolute neutrophilia with a shift to the left was observed.

All other leukocytic counts and differential values were within the normal range.

URINALYSIS

Urine specimens from 4 of the 11 animals were found to have values within normal range.

Protein was detected in the urine of 5 dogs. Four gave a low (2+) reading (30225, 23098, 23068) and one dog (20155C) a high (4+) reading. Later urine specimens obtained from 4 of these dogs failed to confirm the earlier findings.

Granular casts were observed in the urine sediment of 4 dogs (23037, 23068, 23098). Subsequent urine specimens did not contain granular casts.

Dog 23068 had a 3+ urine bilirubin reaction; subsequent samples from this dog failed to show urine bilirubin.

BLOOD CHEMISTRY

Three dogs (30225, 30226, 20155B) had slightly elevated values for BUN (25, 23, 25) and creatinine (1.2, 1.2, 1.5), respectively. Later samples from 2 of the dogs (30225, 30226) gave normal creatinine (0.9, 0.7) and BUN (15, 15) values, respectively.

NECROPSY

No gross genito-urinary lesions were observed in 6 of the animals (30223, 30224, 30225, 20155B, 20155C, 23005). One of these animals (23005) was found to be harboring Dirofilaria immitis.

Kidney

Four animals (30226, 20155A, 23037, 23068) had kidneys whose cortical vessels appeared congested. Small circumscribed whitish foci were seen on the kidney cortex of dog 23010.

Prostate Gland

Several petechial hemorrhages were seen in the prostatic parenchyma of animal 23098. The prostate gland appeared slightly enlarged in 2 male dogs (23037, 30226). The latter animal's prostate also appeared slightly lobulated.

Testes

Testicular abnormalities were seen in 4 dogs. Moderate atrophy of the testis was observed in 2 cases. In one (23068), involvement was unilateral, whereas the other (23098) showed bilateral involvement. One testicle was not located in the scrotum of dog 23010. By dissection the testicle was found external to the inguinal ring in a subcutaneous location. One dog (30225) had bilateral ecchymotic hemorrhages throughout the tunica albuginea.

HISTOPATHOLOGY

A selected summary of histopathologic findings of the kidney and bladder is found in Table 2. A similar summary for the testes, prostate gland and epididymides is compiled in Table 3.

Kidney

A constant finding was infiltration of lymphoreticular cells in the submucosa of the pelvis (Figure 15). The lymphoreticular cells tended to be found in aggregates rather than in random distribution. The size of the aggregate varied from a few cells to large masses which obliterated the normal tissue architecture.

Lymphoreticular cells were noted in perivascular locations throughout the sections examined (Figure 13). The number of vessels showing such changes varied from case to case. Veins, arterioles and capillaries were involved with aggregate size showing wide fluctuation.

Glomerular swelling was a common finding and, in some instances, Bowman's space was completely filled. Hypercellularity of glomeruli was a subtle finding. Differentiation between normal glomerular cells and lymphocytes within the glomerulus could not be made. Glomerular degeneration as denoted by atrophy, pyknosis, fibrosis (Figures 11 and 12) and the presence of an eosinophilic amorphous material within the capsule was sometimes observed.

Fibrosis of either the cortex or medulla was seen in several dogs (Figure 14). The fibrotic response was never generalized.

Limited tubular degeneration denoted by hyaline droplet formation, unevenness of stain, and presence of an eosinophilic amorphous material within the lumen was occasionally seen.

Urinary Bladder

Pathologic changes were observed in the bladder submucosa. Lymphocytic and/or mononuclear cells had accumulated in this tissue (Figure 16). Extensive accumulations of such cells were in groups

rather than randomly distributed. Coagulation necrosis was sometimes seen at the periphery of such aggregates (Figure 16). In one instance a group of lymphoreticular cells was found in the bladder muscularis. Many of the lymphoreticular cells observed were perivascularly located.

A marked disparity in thickness of the submucosal layer was seen. There did seem to be consistency within a related group of animals.

Prostate Gland

Involvement of the prostate gland was always seen. In animals whose lesions were minimal, infiltration and follicular accumulations of lymphoreticular cells were noted in the interstitial connective tissues (Figure 9). Coalescing of follicles was common with destruction of the adjacent glandular lumens (Figures 8 and 10). Fibrosis was noted in areas of marked involvement. In some sections more than 4/5 of the normal histologic architecture was destroyed. Involvement was often lobular. Diseased areas were found adjacent to normal areas being separated only by connective tissue septa.

Epididymides

Histopathologic lesions were observed in all but 2 animals. Accumulations of lymphoreticular cells were noted in the interstitial cell layers (Figure 5). Size of the mass varied from a few cells to rather large aggregates, but in no instance were they seen to cause obliteration or stricture of the tubules. The tubular mucosa showed no abnormalities. Whenever lesions were found in sections taken from the body of the epididymis, lesions were also observed in sections secured from the tail portion. Many of the lymphoreticular aggregates were found to surround blood vessels. Practically all of the smaller lymphoreticular foci were perivascularly located.

Cells were found within the lumen of some tubules. Polymorphonuclear leukocytes, lymphocytes and epithelioid cells were the cell types observed.

The vascular cones in the head of the epididymis were not involved.

Testes

Tissue sections examined in 5 animals showed no histologic abnormality. In 3 of these 5 animals, normal spermatogenesis was observed. The other 2 animals were 85 days of age and, although spermatogenesis through the spermatid stage was noted, spermiogenesis was absent.

In sections from animal 30226 (167 days of age) one observed testicular activity identical to that seen in the 85-day-old animals.

In the other cases, degenerative changes of the seminiferous tubules, as denoted by complete lack of primary and secondary spermatocytes, spermatids, and developing spermatozoa, were seen. Spermatogonial and/or Sertoli cells were the only elements remaining within the tubules (Figure 1). Spermatogonial cells were absent in some of the seminiferous tubules examined. The tubules also contained a large amount of stringy appearing, eosinophilic material. Seminiferous tubule degeneration was found to occur in the absence of any other pathologic change. In some instances degenerative changes occurred in only one testis. In other cases infiltration by lymphoreticular cells into the interstitial layer was found along with seminiferous tubule degeneration. The lymphoreticular cell infiltration may be focal in nature; however, in some instances, focal and diffuse infiltration was noted. Many of the diffuse infiltrations were seen to completely

encircle a seminiferous tubule (Figures 3 and 4). In cases where pathologic changes were severe, seminiferous tubule degeneration, necrosis and fibrosis were observed (Figure 2). An increase in the number of interstitial cells was suggested in these sections. Testicular changes as evidenced by tubular degeneration, lymphoreticular infiltration, necrosis and fibrosis were found to occur unilaterally and bilaterally.

Ductus deferens

Sections from the tail of the epididymis sometimes contained ductus deferens. Lymphoreticular cell accumulations in the submucosa were frequently seen. In sections of ductus deferens taken at various areas, including the ampulla, similar lymphoreticular aggregates were noticed. Similar cells were also seen in the outer adventitia (Figure 6). Accumulations in this area were focal and concentric in appearance. Inflammatory cells (lymphocytes, polymorphonuclear leukocytes and epithelioid cells) were often found within the lumen (Figure 7).

Ureter

Lymphoreticular accumulations were seen in the submucosa of some of the ureter sections examined.

DISCUSSION

Observation and physical examination is of little aid in detection of Brucella canis infection. The detection of an enlarged epididymis or atrophied testicle(s) in an occasional dog should not be considered pathognomonic for this infection. Testicular atrophy could result from other clinical entities as Sertoli cell tumor, chronic bacterial infection or hormonal dyscrasias.

Little correlation between infection and hematologic results was found. The reduction in hemoglobin, packed cell volume and erythrocytic count observed in the 4 neonates is a normal physiological anemia observed in all puppies, regardless of sex, during the first weeks of life (Schalm, 1965). This same group of animals possessed a neutrophilia at birth. Since the animals were known to be bacterially contaminated only with Brucella canis the neutrophilia cannot be attributed to any other bacterial infection. It has been noted by the author that initial blood samples taken from many axenic pups have given absolute neutrophilic values far in excess of accepted normal values. Typically such values rapidly decrease to normal ranges within the first or second week of life.

The rise in absolute lymphocytes to 7000-7750/cu mm of blood at 112 days of age and the persistence of the lymphocytosis until euthanasia at 167 days is an interesting phenomenon. Since one of the primary infiltrating cells seen in histopathologic sections is the lymphocyte, an elevation in the number of circulating lymphocytes might

be expected. Since a lymphocytosis was observed in only 2 of 10 animals monitored, a valid conclusion as to direct association with the disease cannot be drawn. A more systematic sampling of the group might have yielded similar findings. Experimentally induced infection with consistent hematologic monitoring would possibly clarify the significance of the finding.

The elevated leukocytic counts observed in animals 20155A and 20155B at 94 days of age were consistent with clinical symptoms and are considered unrelated to the brucellosis infection.

The neutrophilia recorded at time of euthanasia in animals 20155A and 23037 cannot be explained.

Urinalyses and blood chemistries showed no consistent patterns. One urine specimen from dog 20155C gave a 4+ reaction for protein, a sample procured 29 days later yielded normal values, as did blood urea nitrogen and creatinine determinations. Histopathologic examination of the animal's kidneys showed pelvic infiltration and perivascular cuffing. Significance of the finding as it relates to this disease is held to be of little import. Due to the variations encountered between animals, and in the same animals, the urinalyses data are, at best, inconclusive. Again monitoring experimentally infected animals would be of value as would observation of naturally infected animals for more extended periods of time.

Histopathologic studies indicate that the primary response is by the reticuloendothelial system. Other studies have shown that lymph nodes in many instances are hyperplastic (Moore, 1967; Carmichael, 1967). Increased lymphoid activity within the spleen and liver has also been suggested (Moore, 1967).

Lymphoreticular infiltration of the prostate gland with subsequent loss of normal glandular architecture was the most striking finding. The degree of reaction in all but the 2 youngest animals was moderate to extensive in severity. The 2 youngest animals (87 days of age) did show slight infiltration of the interstitial tissue by lymphoreticular cells. The absence of severe lesions in these animals might be the result of incomplete glandular development at this age. In the affected animals a definite impairment of prostatic function would be expected.

Infiltration of the renal pelvis submucosa by lymphoreticular cells was another constant finding. The degree of involvement was not as extensive as that observed in the prostate gland.

The occurrence of glomerular swelling, and in some instances degenerative changes, were frequently noted. This finding could be totally unrelated to the disease syndrome; however, it is not remiss to associate a persistent bacteremia of chronic duration in conjunction with persistent antibody titer with the glomerular changes. Fluorescent antibody techniques would be of great aid in attempting to clarify this finding as it related to the disease. Fibrotic changes seen in the kidney cannot be conclusively associated with this disease on the basis of its occurring in only 3 of 12 dogs.

Lesions within the bladder were confined in all but one instance to the submucosa. In the one exception, the lesion was located between muscle bundles of the muscularis layer but was similar in nature to those observed in the submucosa. The lesions were seen in only 4 animals but are felt to be germane to the disease entity since this is the primary cell type noted in other lesions. There was no definite correlation between the finding of bladder lesions and recovery of

Brucella canis from the bladder urine samples.

Lesions were observed much more frequently in the epididymides than in the testes. In no instance were lesions observed in the testis without lesions being observed in the associated epididymis. It was observed that only one epididymis could be involved. Aggregations of lymphoreticular cells were not as common in the testicle as in other organs. In some instances, degenerative changes within the seminiferous tubules were noted in the absence of any other pathologic findings. This finding is not consistent with pathologic lesions observed in other tissues. Progression of testicular lesions appears to be lymphoreticular cell infiltration, necrosis of seminiferous tubules and replacement by fibrous elements in a final sequel.

The only 2 animals in which pathologic lesions were not observed in the epididymides were the 87-day-old litter mates. The incompleteness of sexual development at this age may account for this finding. It appears that the epididymides are one of the primary tissues affected in the disease syndrome. Histopathologic findings suggest that testicular involvement is by direct extension of the disease process from the associated epididymis.

The culturing of Brucella canis from urine specimens procured by bladder puncture is in contradiction to the findings reported by Carmichael (1967). This observation in 8 out of 12 animals examined appears to be significant. Constant shedding of the organism in the urine may be an important means by which the disease is disseminated. This finding in males is contrary to that observed in females (Moore, unpublished data). The seemingly contradictory results may be accounted for by the close proximity of the prostate gland and ductus deferens to the bladder in the male.

It was observed that 4 of the infected dogs ceased to be bacteremic during the course of the study. There was a concomitant decrease in serum agglutination titer. Similar findings were recently presented by Carmichael (1967). Indications are that disappearance of a bacteremia does not signify recovery from the disease. Animal 23068 was euthanatized 4 weeks after loss of a bacteremia. Bacteriologic culture of the prostate gland isolated Brucella canis.

In further observations on the 3 other animals that ceased to show a bacteremia, physical changes of the epididymides and testicles were observed. Testicular abnormalities were also observed in 4 other infected dogs at necropsy. These physical alterations are considered consistent with histopathologic findings observed in the same organs of other infected dogs.

An interpretation of bacteriologic blood culturing studies suggests that loss of a bacteremia is associated, in some dogs, with chronicity of infection. If the actual date of infection was known in all of the diseased dogs studied a definite interpretation would have been possible.

In no instance has an infected dog been observed to completely lose its agglutination titer. This observation, in conjunction with the observation that a marked drop in agglutination titer accompanies the loss of a bacteremia, forces one to reexamine the status of the 4 clinically normal dogs with agglutination titers. Are these 4 individual animals in a chronic state of infection? Direct evidence in support or rejection of the question is currently lacking. Limited studies by the author on female Beagles that also possessed agglutination titer in the absence of a bacteremia may be enlightening (Moore,

1967). Several nonbacteremic females possessing agglutination titer were euthanatized and tissues cultured. Other females with agglutination titers were bred, cultured for bacteria repeatedly during pregnancy, recultured at parturition and postparturiently. The offspring resulting from these pregnancies were monitored bacteriologically and serologically. All bacteriologic cultures were negative. The offspring possessed no Brucella canis agglutination antibody, further indicating lack of exposure or infection.

The 4 clinically normal males possessing agglutinating antibody also bred bitches during the course of this study. Conception rates and whelping data on these bitches are considered to be normal. Emphasis on this latter finding should not be stressed, since an infected male dog (23037) was allowed to breed several noninfected bitches. All whelped normal, viable puppies.

Until more extensive work is done on the direct relationship of agglutination titer and actual infection, an animal cannot be considered infected with Brucella canis on the sole basis of possessing an agglutination titer. Because of this unresolved finding, a sound prophylactic breeding program must require that males possessing an agglutination titer for Brucella canis be withheld from breeding. Adherence to this recommendation is of twofold benefit.

1. Elimination of what could prove to be a rapid means of disseminating the disease.
2. A feasible method of determining total breeding colony status would be utilization of males with no agglutination titer, if frequently monitored serologically.

Since infected dogs have been observed to become abacteremic, one cannot determine that an animal, on the basis of a negative bacteriological blood culture, is free of Brucella canis infection.

In instances where a serologically and bacteriologically negative dog has been found to later acquire the disease, a bacteremia and rapid rise in agglutination titer to 400 (usually 800) was observed (Moore, unpublished data). Carmichael (1967) recorded similar findings in experimentally infected dogs. It appears reasonable to conclude that any isolated animal with no agglutinating antibody, in 2 successive serums collected 3 weeks apart, is free of Brucella canis infection.

SUMMARY AND CONCLUSIONS

A study was conducted on 27 male dogs, 17 of which were naturally infected with Brucella canis, to attain insight as to the disease process. Major effort was expended on diagnostic means of detection and the disease's effect on the urogenital system. The following were considered the most pertinent findings:

1. Brucella canis was capable of in utero infection.
2. A bacteremic phase of variable duration was observed in all infected animals.
3. All infected animals possessed an agglutination titer.
4. The strength of the agglutination titer decreased in animals observed to become abacteremic but in no instance was an infected animal found to entirely lose its agglutination titer.
5. A definite correlation between possession of an agglutination titer and presence of infection could not be made.
6. Some infected males developed a clinically observable epididymitis and/or regression of testicle size. The changes were noticed to be either unilateral or bilateral.
7. Hematologic, urologic and blood chemistry studies revealed no consistent abnormalities.

8. Bladder urine specimens obtained from 12 dogs were found to harbor Brucella canis in 8 instances.
9. Histologic examination of tissues found the primary cellular response to be of reticuloendothelial origin.
10. The prostate gland was always affected; almost total loss of normal glandular structure was a common finding in adult dogs.
11. Partial or total sterility due to either aspermia or destruction of seminiferous tubules and replacement by fibrous tissue was frequently observed either bilaterally or unilaterally.
12. Pathologic changes were commonly seen in the renal pelvis, glomeruli, and around smaller blood vessels in the kidney. Significance of these lesions as they affect renal function could not be ascertained.
13. Lymphoreticular infiltration was observed in the epididymides, urinary bladder, ureters and ductus deferens.

Table 1. Results of serologic and bacteriologic procedures for detection of Brucella canis at time of necropsy

Dog No.	Age at Necropsy (days)	Age When Infected	Agglutination Titer	Bacteriology					
				Blood	Epididymis Testes	Prostate Gland	Bladder Urine	Spleen	Kidney
30223	85	Congenital	400	+	+	+	Negative	+	---
30224	85	Congenital	800	+	+	---	Negative	+	+
30225	167	Congenital	800	+	+	+	+	+	+
30226	167	Congenital	800	+	+	---	+	+	+
20155A	269	Neonate***	800	+	+	+	+	+	---
20155B	312	Neonate***	800	+	+	---	Negative	+	---
20155C	361	Neonate***	800	+	Negative	+	Negative	+	+
23005	Adult + 8*	Adult	400	+	+	+	+	+	---
23010	Adult + 14*	Adult	800	+	+	+	+	+	+
23037	Adult + 64**	Adult	800	+	+	+	+	+	+
23068	Adult + 79*	Adult	200	Negative	Negative	+	+	Negative	---
23098	Adult + 103*	Adult	800	+	+	+	+	---	---

* - Number of days elapsed since original isolation; found infected at time of initial culture - duration not known.

** - Animal not infected at time of initial culture, number of days elapsed since original isolation indicative of length of infection.

*** - Animals not cultured until 200 days of age - assume neonatal infection since Brucella canis isolated from litter mate as neonate. Dam also yielded Brucella canis in blood culture and vaginal swab culture.

--- - Not done.

Table 2. Summary of selected histopathologic findings: urinary system

Dog No.	Kidney			Bladder
	Pelvic Infiltration	Perivascular Cuffing	Fibrosis	Submucosal Infiltration
30223	slight	slight	none	slight
30224	slight	slight	none	slight
30225	extensive	slight	present	none
30226	slight	moderate	none	extensive
20155A	moderate	moderate	present	none
20155B	slight	moderate	none	slight
20155C	moderate	slight	none	none
23005	slight	moderate	none	none
23010	slight	moderate	present	none
23037	extensive	slight	none	none
23068	slight	extensive	none	none
23098	extensive	extensive	none	none

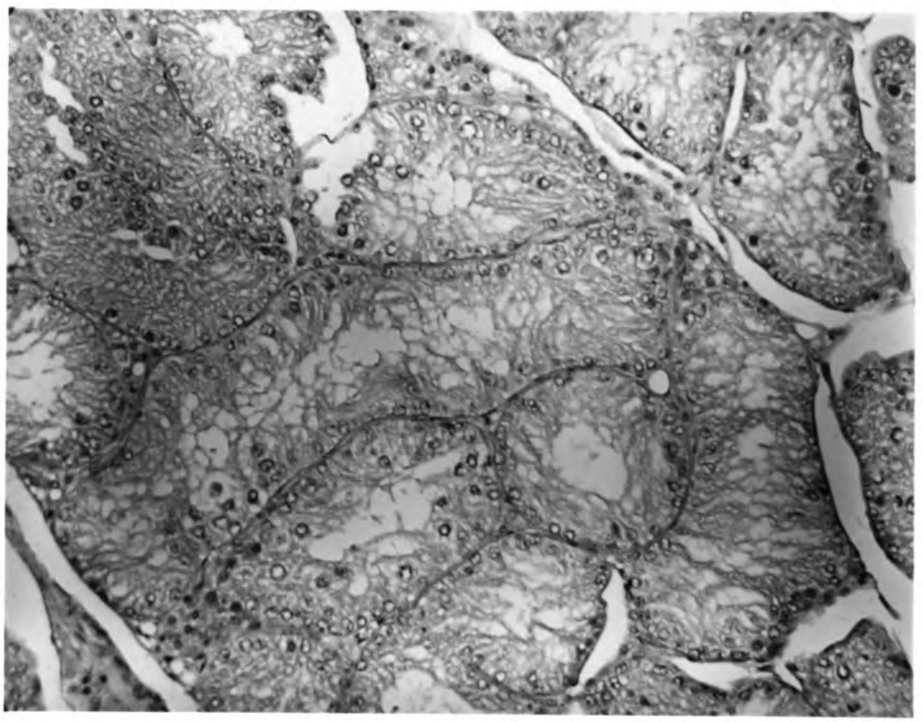
Table 3. Summary of selected histopathologic findings: reproductive system

	Prostate Gland	Testes				Epididymis
Dog No.	Interstitial Infiltration	Spermatogenesis	Seminiferous Tubule Degeneration	Lymphoreticular Accumulation	Fibrosis	Lymphoreticular Accumulation
30223	slight	none	none	mpme	none	none
30224	slight	none	none	none	none	none
30225	moderate	none	bilateral	none	none	bilateral
30226	extensive	none	none	none	none	bilateral
20155A	extensive	yes*	none	none	none	yes*
20155B	extensive	yes*	none	none	none	yes*
20155C	extensive	unilateral	bilateral	bilateral	none	bilateral
23005	moderate	bilateral	none	none	none	unilateral
23010	moderate	bilateral	unilateral	none	none	unilateral
23037	moderate	bilateral	none	none	none	unilateral
23068	moderate	unilateral	unilateral	unilateral	unilateral	unilateral
23098	extensive	none	bilateral	bilateral	bilateral	bilateral

* One testicle or epididymis examined

Figure 1. Testicle, Dog 23010. Aspermia and seminiferous tubule degeneration. X165.

Figure 2. Testicle, Dog 23098. Necrosis and loss of seminiferous tubules with replacement by fibrous elements and inflammatory exudate. X165.



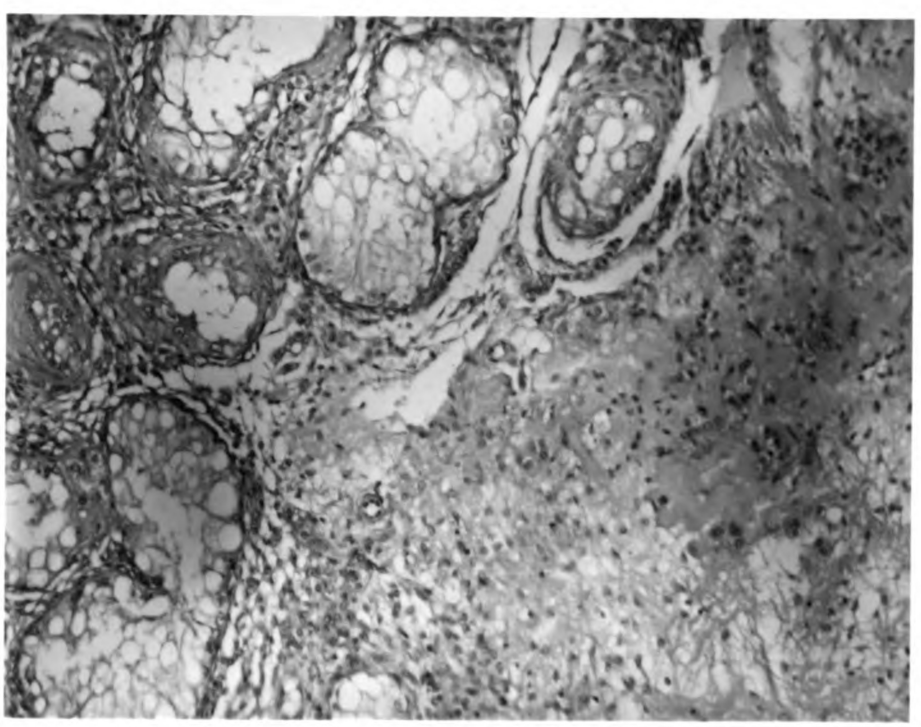
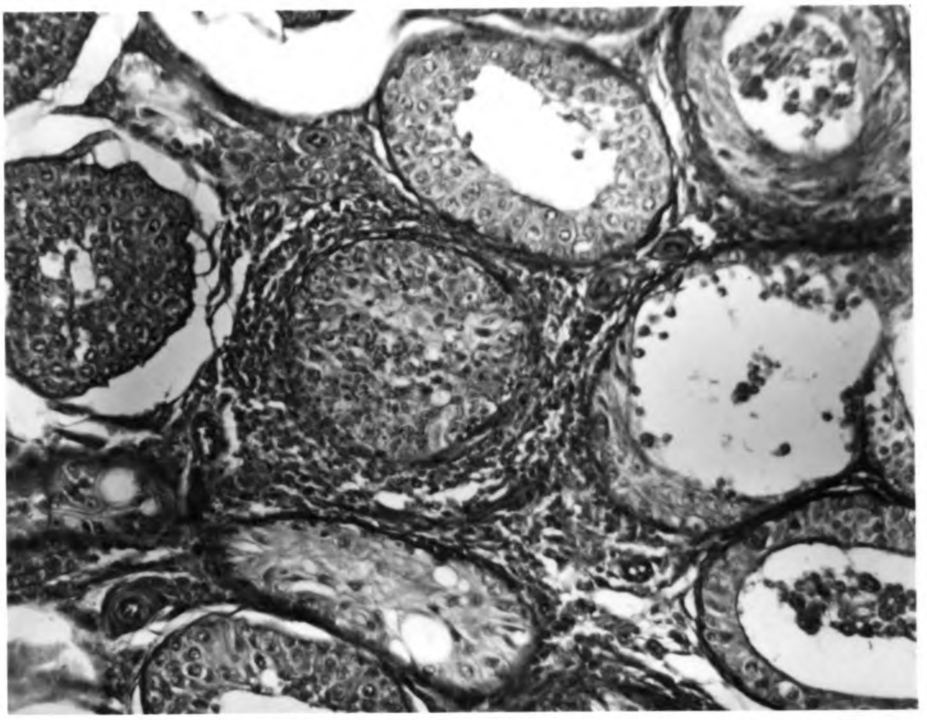


Figure 3. Testicle, Dog 20155C. Interstitial infiltration with lymphoreticular cells surrounding seminiferous tubule. Few lymphoreticular cells within the seminiferous tubule. X165.

Figure 4. Testicle, Dog 20155C. X413.



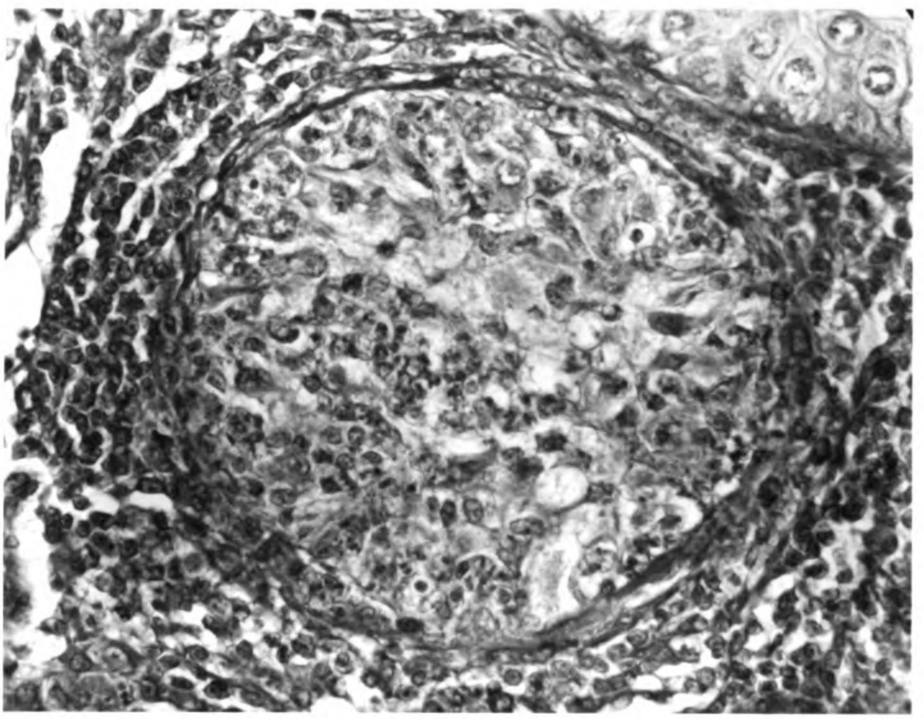
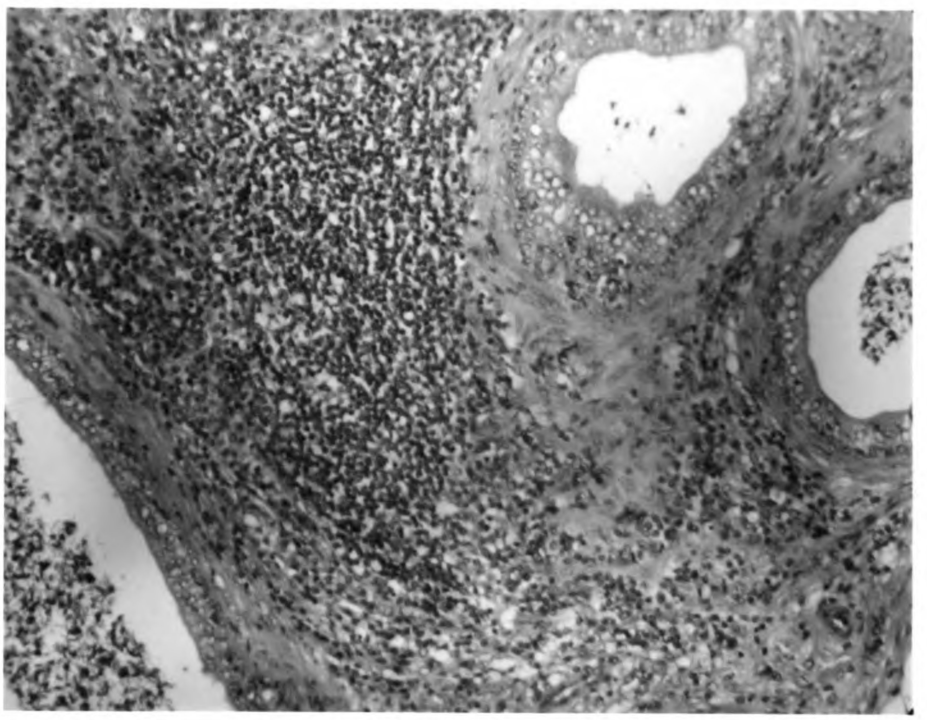


Figure 5. Epididymis, Dog 20155B. Interstitial infiltration and aggregation of lymphoreticular cells. X165.

Figure 6. Ductus deferens, Dog 30225. Concentric accumulation of lymphoreticular cells in the ductus wall. X165.



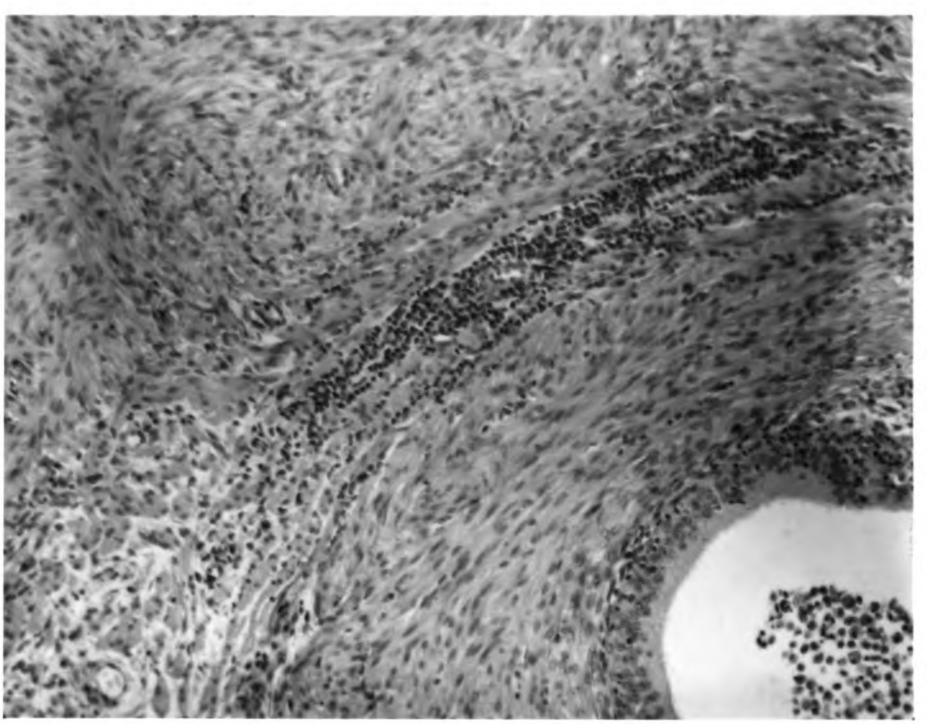
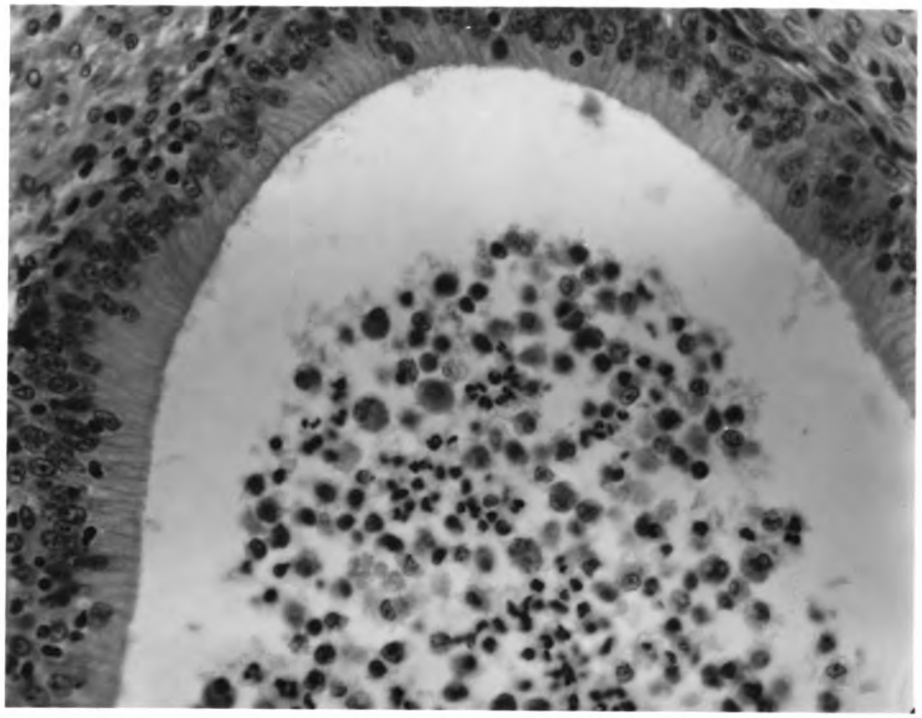


Figure 7. Ductus deferens, Dog 30225. Polymorphonuclear leukocytes, mononuclear cells in lumen of ductus deferens. X413.

Figure 8. Prostate gland, Dog 23005. Lymphoreticular cells infiltrating the interstitial cell layer. Inflammatory cells within a gland's lumen. X413.



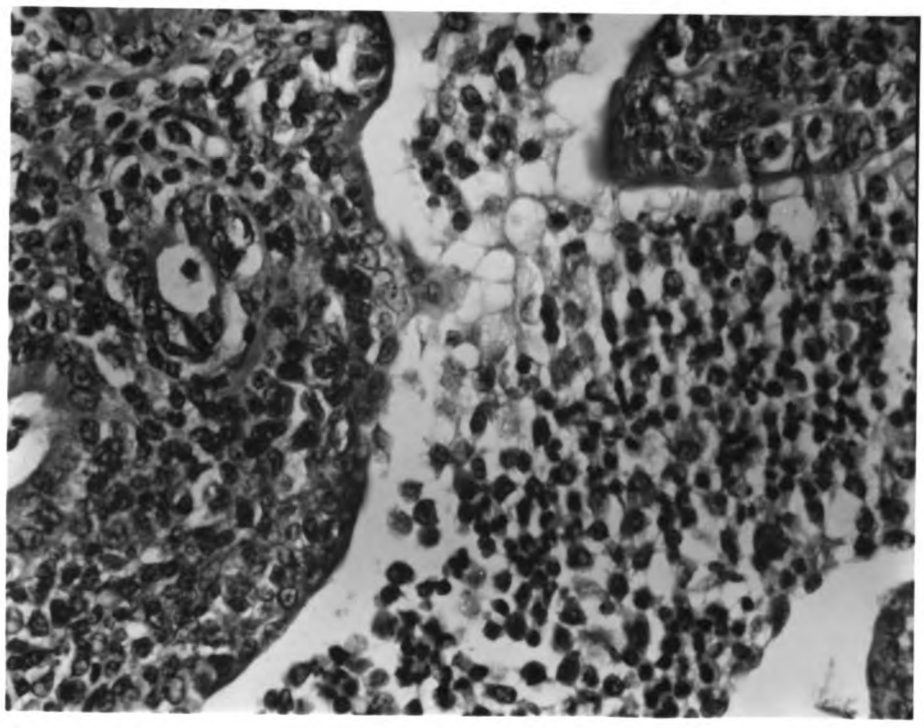
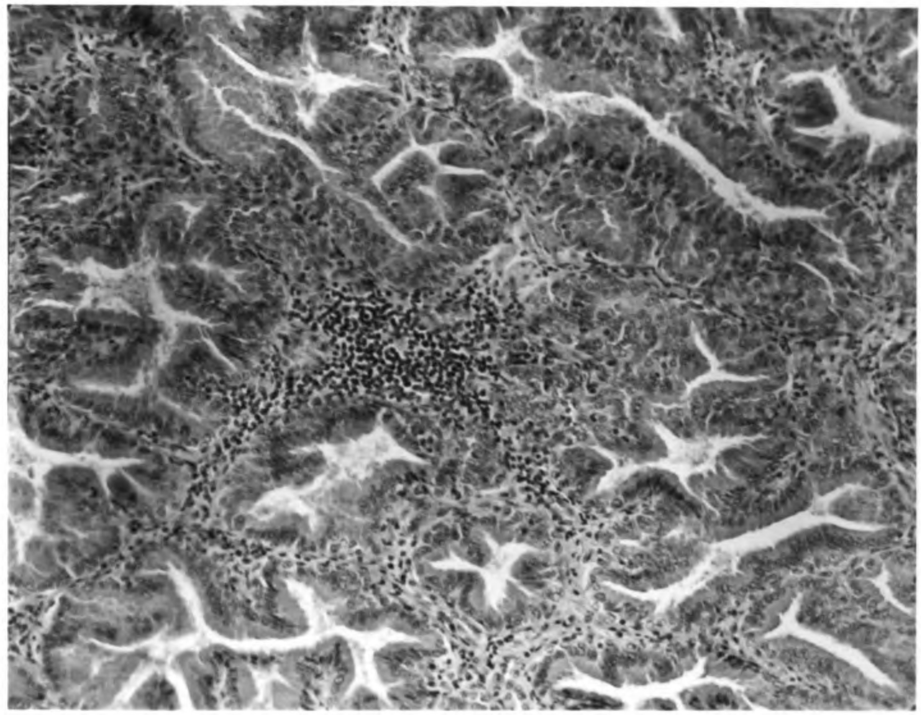


Figure 9. Prostate gland, Dog 23068. Early accumulations of lymphoreticular cells in the interstitial cell layer of prostate gland. X165.

Figure 10. Prostate gland, Dog 23005. Area of extensive infiltration of lymphoreticular cells with loss of glandular elements. X165.



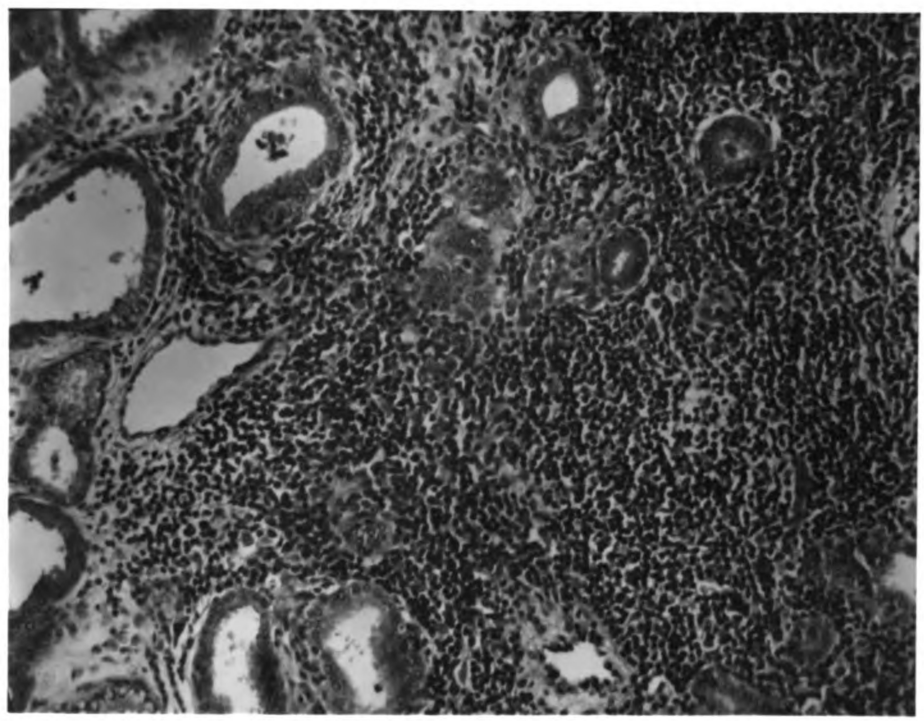
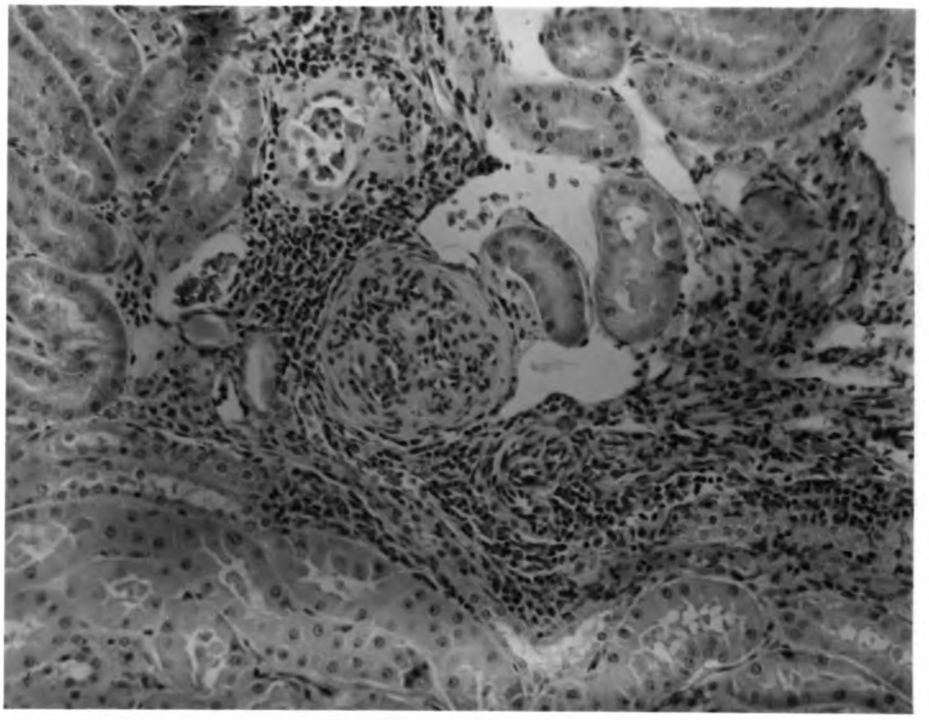


Figure 11. Kidney, Dog 23068. Blood vessel and fibrosed glomerulus surrounded by lymphoreticular cells. X165.

Figure 12. Kidney, Dog 23068. X413.



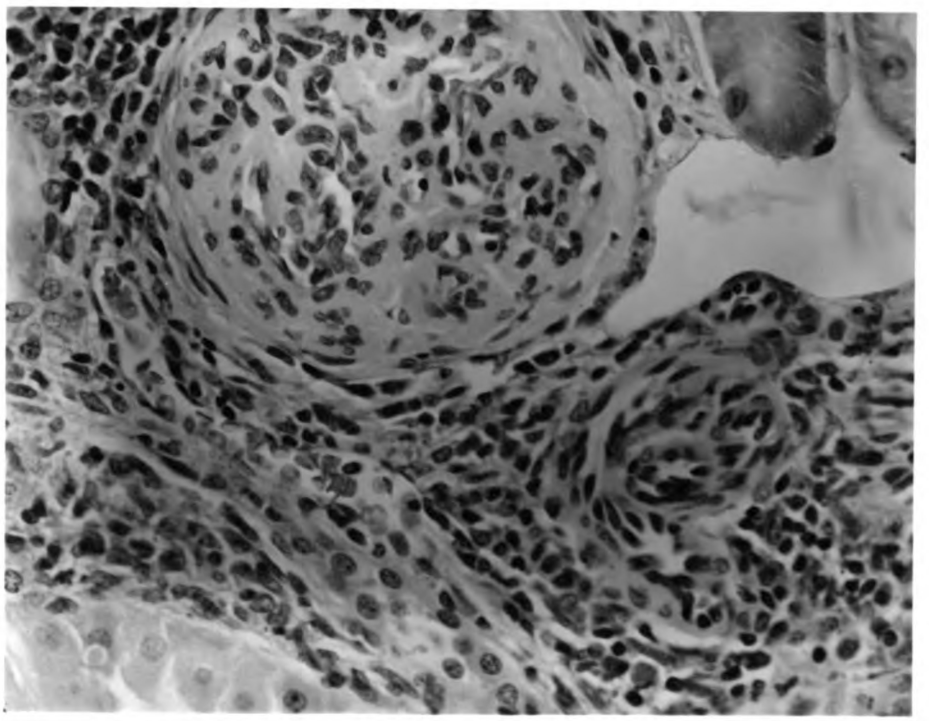
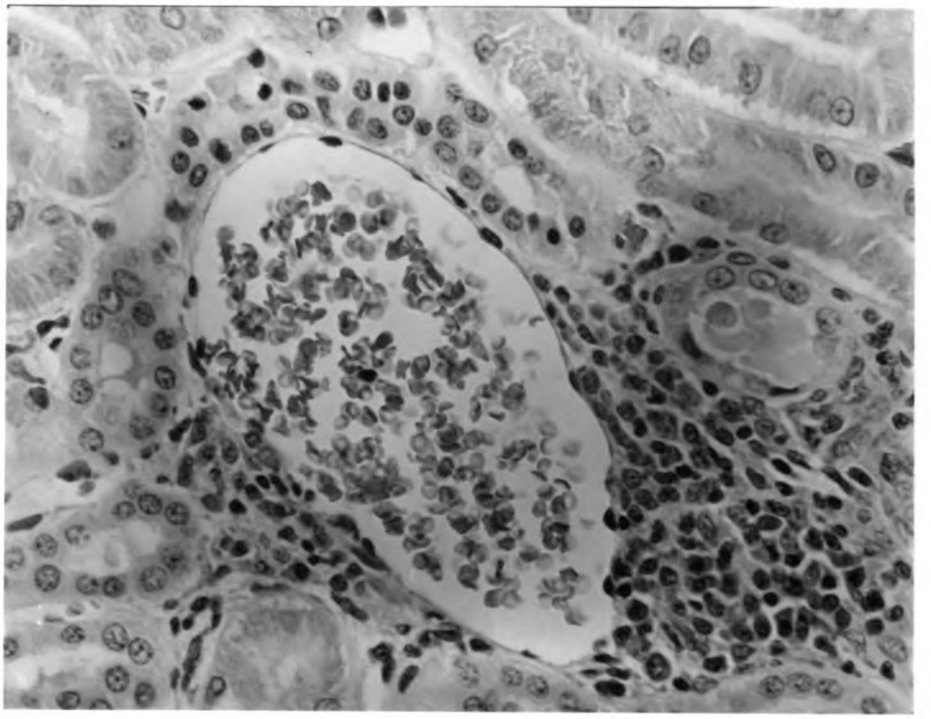


Figure 13. Kidney, Dog 23098. Accumulation of lymphoreticular cells adjacent to a blood vessel in the cortex. X413.

Figure 14. Kidney, Dog 30225. Fibrosed area in cortex. X165.



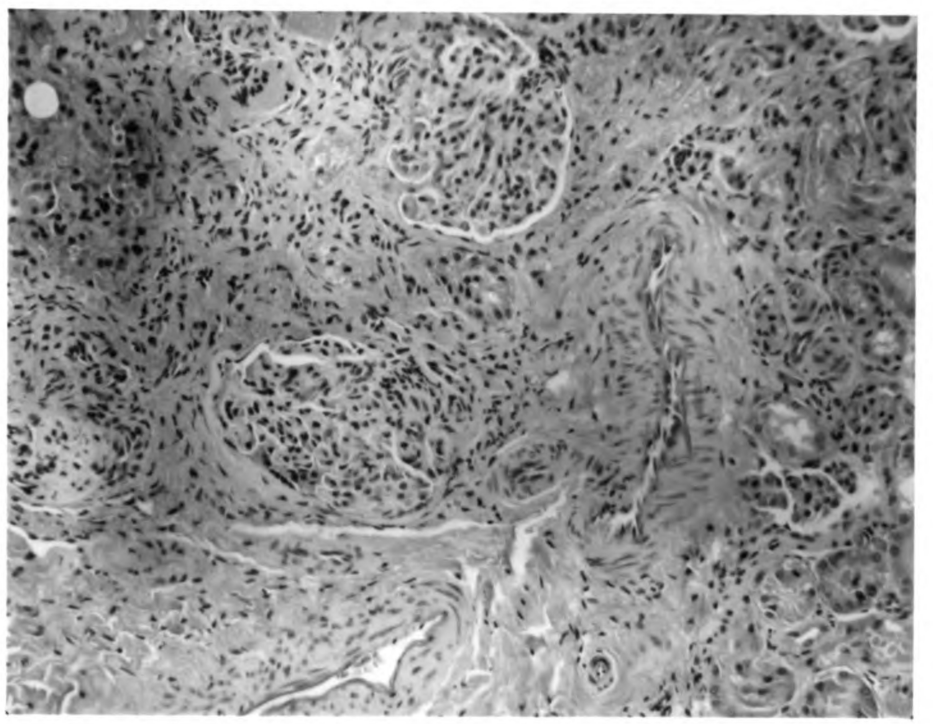
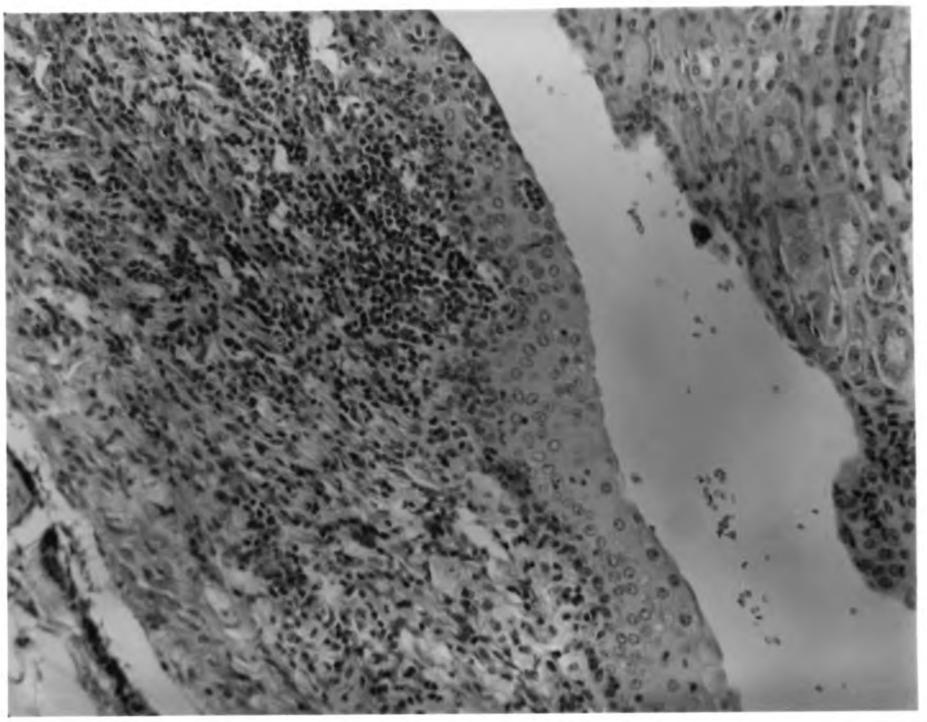
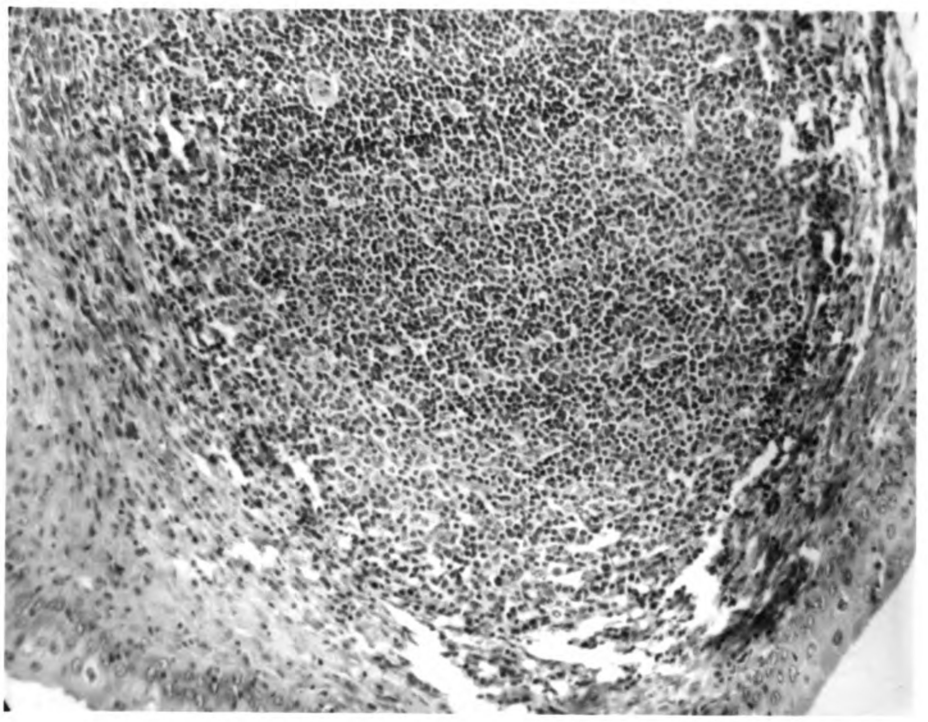


Figure 15. Kidney, Dog 23098. Lymphoreticular cells in submucosa of the renal pelvis. X165.

Figure 16. Urinary bladder, Dog 30226. Large lymphoreticular aggregate in the submucosa with necrosis observed at the aggregate periphery. X165.





Appendix 1. Table of dogs utilized in bacteriology and serology study

Dog No.	Status	Number of Samples Tested Serologically	Number of Blood Samples Cultured Bacteriologically
23001	Normal	5	3
23003	Normal	5	4
23004	Normal	3	4
23006	Normal	4	4
23008	Normal	4	4
23014	Normal	4	3
23015	Normal	4	4
23028	Infected	3	7
23032	Infected	4	6
23035	Infected	4	4
23039	Normal	4	3
23049	Normal	3	3
23065	Normal	5	3
23068	Infected	4	3
23075	Infected	4	2
23098	Infected	3	3

Appendix 2. Table of dogs utilized in clinical pathology and pathologic studies

Dog No.	Status	Number of Hematological Samples	Number of Urinalyses	Number of Blood Chemistries	Necropsy and Histopathology
20155A	Infected	2	---	---	X
20155B	Infected	2	1	2	X
20155C	Infected	2	2	1	X
23005	Infected	---	---	---	X
23010	Infected	---	---	---	X
23028	Infected	---	2	---	---
23032	Infected	---	1	---	---
23035	Infected	---	1	---	---
23037	Infected	2	2	1	X
23068	Infected	4	2	1	X
23075	Infected	---	---	---	---
23098	Infected	3	3	1	X
23100	Infected	---	1	---	---
30223	Infected	7	---	---	X
30224	Infected	4	---	---	X
30225	Infected	9	2	2	X
30226	Infected	8	3	2	X

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