3.2.7 Proliferative Kidney Disease

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A. Name of Disease and Etiological Agent

Proliferative kidney disease (PKD) is caused by the PKX myxozoan, which was recently described as *Tetracapsuloides bryosalmonae* (syn. *Tetracapsula bryosalmonae*, *Tetracapsula renicola*) (see Canning et al. 2002).

B. Known Geographical Range and Host Species of the Disease

1. Geographical Range

The disease has been reported in major rainbow trout producing countries throughout Europe. In North America, PKD has been reported in the United States from California, Idaho, Washington, and in Canada from British Columbia and Newfoundland.

2. Host Species

Natural infections of PKX have been reported in rainbow trout and steelhead *Oncorhynchus mykiss*, cutthroat trout *Oncorhynchus clarki*, chinook salmon *Oncorhynchus tshawytscha*, coho salmon *Oncorhynchus kisutch*, Atlantic salmon *Salmo salar*, brown trout *Salmo trutta*, arctic char *Salvelinus alpinus*, grayling *Thymallus thymallus*, and pike *Esox lucius*. Infections have been observed in captive and free-living populations. Kokanee salmon *Oncorhynchus nerka* and chum salmon *Oncorhynchus keta* have been experimentally infected.

Bryozoans are required alternate host in the life cycle. Species of bryozoans that have been identified as host for PKX include *Plumatella emarginata* and *Fredericella sultana* (Canning et al. 1999; Longshaw et al. 1999).

C. Epizootiology

Proliferative kidney disease is caused by presporogonic stages of the parasite that infect the kidney interstitium and other well-vascularized organs. Incomplete sporulation occurs in kidney tubules. Clinical disease is primarily observed in young-of-the-year fish when summer water temperatures are greater than 12^{o}C. Previously uninfected yearlings are susceptible, whereas survivors show strong resistance to reinfection. Infections are detected approximately one month after exposure and clinical

disease follows about 3 to 4 weeks later. Mortality is chronic and extremely variable (5-90%). High mortalities are often associated with secondary infections of *Aeromonas salmonicida*, *Flexibacter columnaris*, or *Ichthyophthirius multifilis*. Recovery from PKD begins to occur in an infected population at about 12 weeks post-exposure and most fish that survive recover after 20 weeks.

D. Disease Signs

1. **Behavioral Changes Associated with the Disease**
   Fish with PKD are often lethargic and dark.

2. **External Gross Signs**
   Affected fish exhibit exophthalmia, lateral body swelling, and a distended abdomen. Gills are pale due to anemia.

3. **Internal Gross Signs**
   Internal gross signs are ascites and enlargement of the spleen and the posterior or entire kidney. The kidney is often grey and mottled and uniformly enlarged, and the kidney capsule is often distended ventrally in a corrugated pattern (Figure 1, Figure 2, and Figure 3).

4. **Histopathological Changes Associated with the Disease**
   Histopathological examination shows the kidney interstitium to be the primary site of infection, where the parasite evokes a chronic interstitial nephritis. The initial stage of the disease is characterized by hematopoietic hyperplasia. This is followed by diffuse, chronic inflammation. Coalescing whorls of inflammatory cells, primarily macrophages, surround the parasites (Clifton-Hadley et al. 1984, 1987; Smith et al. 1984; MacConnell et al. 1989). Parasites also infect blood vessels, where they adhere to vessel walls, occlude vessels, and evoke a necrotizing vasculitis. Well-vascularized extrarenal organs (e.g. gills, liver, spleen, and pancreas) also are infected and exhibit similar histologic changes as found in the kidney (Figure 4).

Figure 1. Macroscopic changes of PKD in rainbow trout. Note bloody ascites, enlarged spleen and pale gills. Kidney shows moderate enlargement in posterior region.

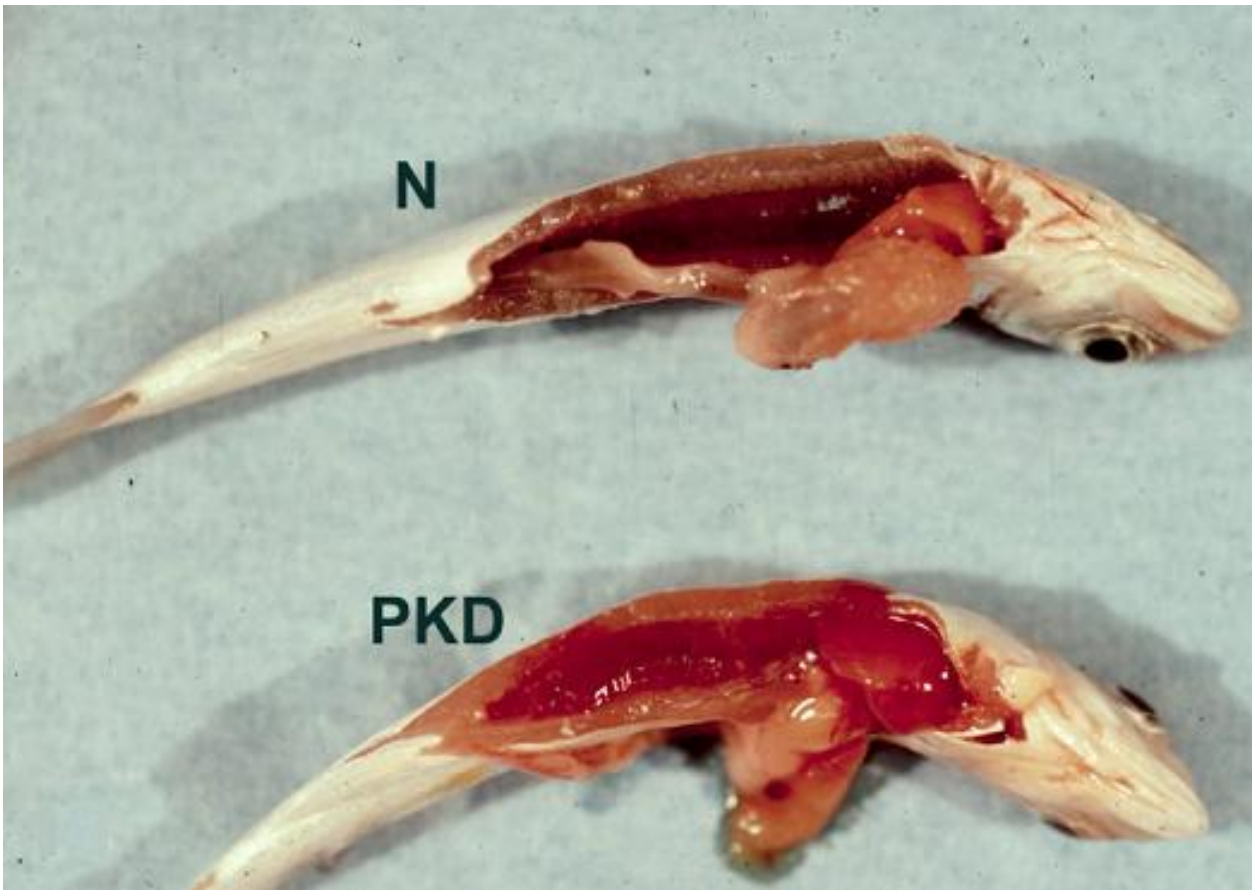


Figure 2. Chinook salmon with PKD, compared to normal (N). Note uniformly enlarged kidney in lower fish with PKD.

Figure 3. Macroscopic changes of PKD in cutthroat trout. Note enlarged spleen, kidney, and pale gills.

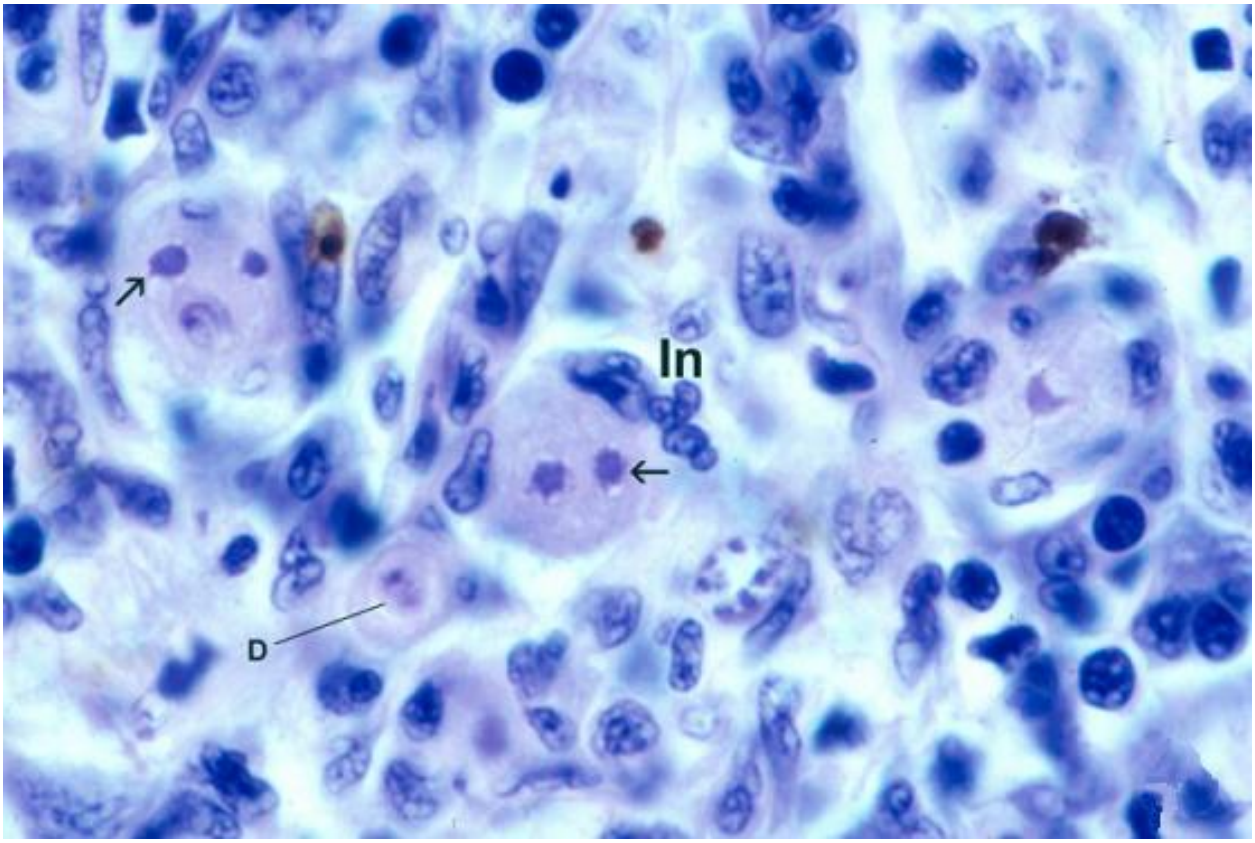


Figure 4. PKX cells in the kidney interstitium in histological section stained with H&E. Arrows = primary cell nuclei, D = daughter cell, In = inflammatory cells surrounding parasites.

E. Disease Diagnostic Procedure

1. Presumptive Diagnosis

The PKX parasite can be presumptively identified in Giemsa-stained imprints of the posterior kidney or spleen (Klontz and Chacko 1983). In these preparations, PKX parasites are round, 10 to 20 µm in diameter, and have a light staining, vacuolated cytoplasm (Figures 5 and 6). One to seven secondary (daughter) cells are found within the cytoplasm of the primary (mother) cell. Intensely stained inflammatory cells often are attached to and surround the parasite. Wet mounts may also be used for presumptive diagnosis, in which the PKX parasite occurs within the kidney interstitium. PKX organisms can be differentiated from host cells by the presence of a distinctive cell membrane and small nuclei (Figure 7).

2. Confirmatory Diagnosis

Confirmatory diagnosis is based on histological examination of tissue sections stained with hematoxylin and eosin (Figure 4) or tissue imprints stained with the GS-1 specific lectin as described by Hedrick et al. (1992). For histology, the principal diagnostic feature of PKD is the presence of the PKX parasites in the renal interstitium. The PKX parasite is amoeboid, 5 to 20µm in diameter, and has a foamy, eosinophilic cytoplasm. The primary cell contains 1 to 3 distinctive nuclei, which are characterized by the presence of a large, intensely stained, eosinophilic endosome (nucleolus). One to seven spherical, dense daughter (secondary) cells are present in most of the parasites. Some of the secondary cells contain tertiary cells. Inflammatory cells are attached to and surround many of the PKX parasites (Figure 4).

Detection of PKX organisms in tissue imprints stained with lectin (Hedrick et al. 1992) is as follows: a) Kidney or spleen pieces tissues are blotted on clean paper tissues to remove excess blood and imprints are made on clean slides. b) After air drying for 30 minutes, imprints are fixed in acetone ethanols (60:40) at -20°C for 10 minutes. c) Slides are air dried and stained immediately or stored at -70°C under desiccated conditions.

The biotinylated GS-1 lectin from *Griffonia simplicifolia* (L-37359 Sigma, St. Louis) is suspended in 0.01M phosphate buffer pH 6.8 at 5-10 µg/L. Aliquots are place on the imprints and incubated for 1 hour at 25°C. Slides are rinsed three times in PBS and fluorescine at 30 µg/L is placed over the imprints. Slides are again incubated in a moist chamber at 25°C for 30 minutes. Slides are rinsed three times, carefully blotted dry, and mounted with a coverslip using a drop of a mixture containing 1 part 0.1M N-2-hydroxy-ethylpiperazin-N'-2-ethanesulfonic acid (HEPES) pH 8.0 and 9 parts glycerol. The specimens are observed under a UV microscope.

PKX cells stain brilliant apple green with a granular cytoplasm (Figure 8). Nuclei and daughter cells appear as dark, subspherical bodies within the cytoplasm of the PKX organism. This lectin has also been used to identify PKX in tissue sections (Figure 9) (Marin de Mateo et al. 1996).

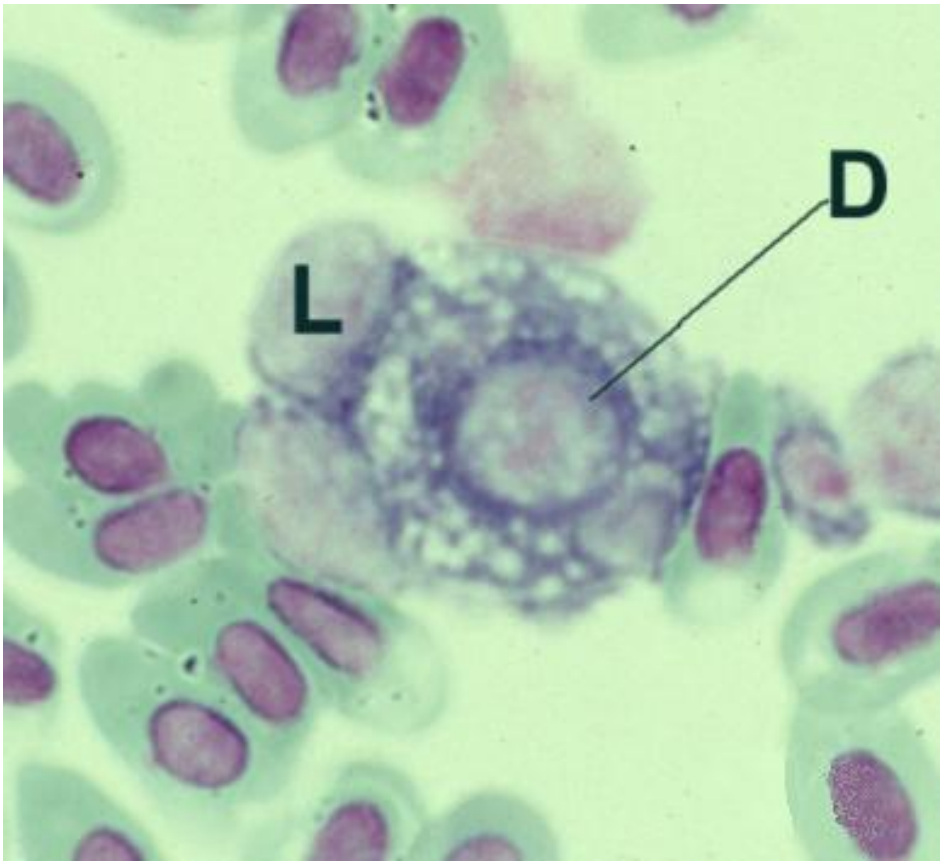
Monoclonal antibodies specific to PKX have been also used to visualize parasites in tissue sections (Adams et al. 1992; Marin de Mateo et al. 1996).

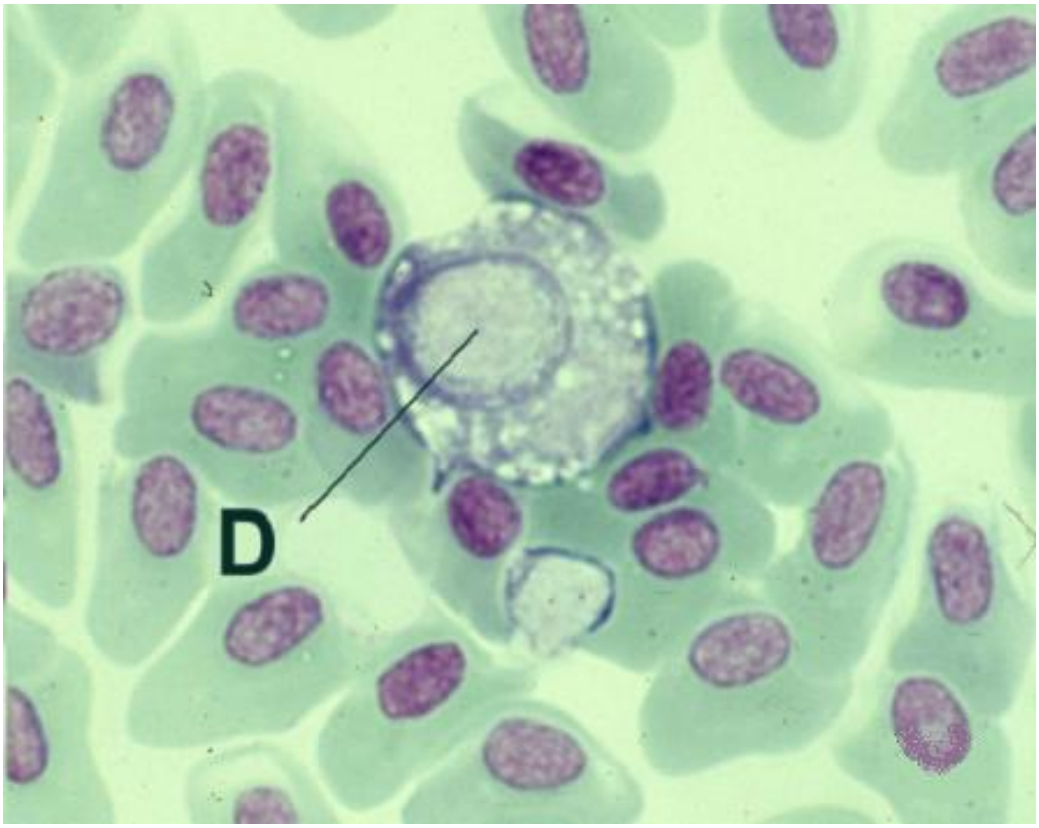
F. Procedures for Detecting Subclinical Infections

Necropsy of apparently healthy fish that are examined during the summer months from waters in which the parasite is enzootic will frequently reveal renal and splenic hypertrophy. Histological examination of the kidney will often reveal the parasite and the typical tissue reaction, although the latter is usually milder than that found in clinically affected fish.

Examination of wet mounts with phase microscopy or kidney imprints is also effective for detecting subclinical infection.

PCR are also useful for detecting light infections. PCR tests using small subunit ribosomal DNA primers were reported by Kent et al. (1998). The most sensitive and specific primer sets appear to be PKX 3f and 4r or PKX 5f and 6r (Kent et al. 1998; Morris and Adams 2002).





Figures 5 and 6. PKX cells in Giemsa imprints. Note lymphocytes (L) surronding parasite. PKX cells has foamy cytoplasm and contains daughter cells (D).

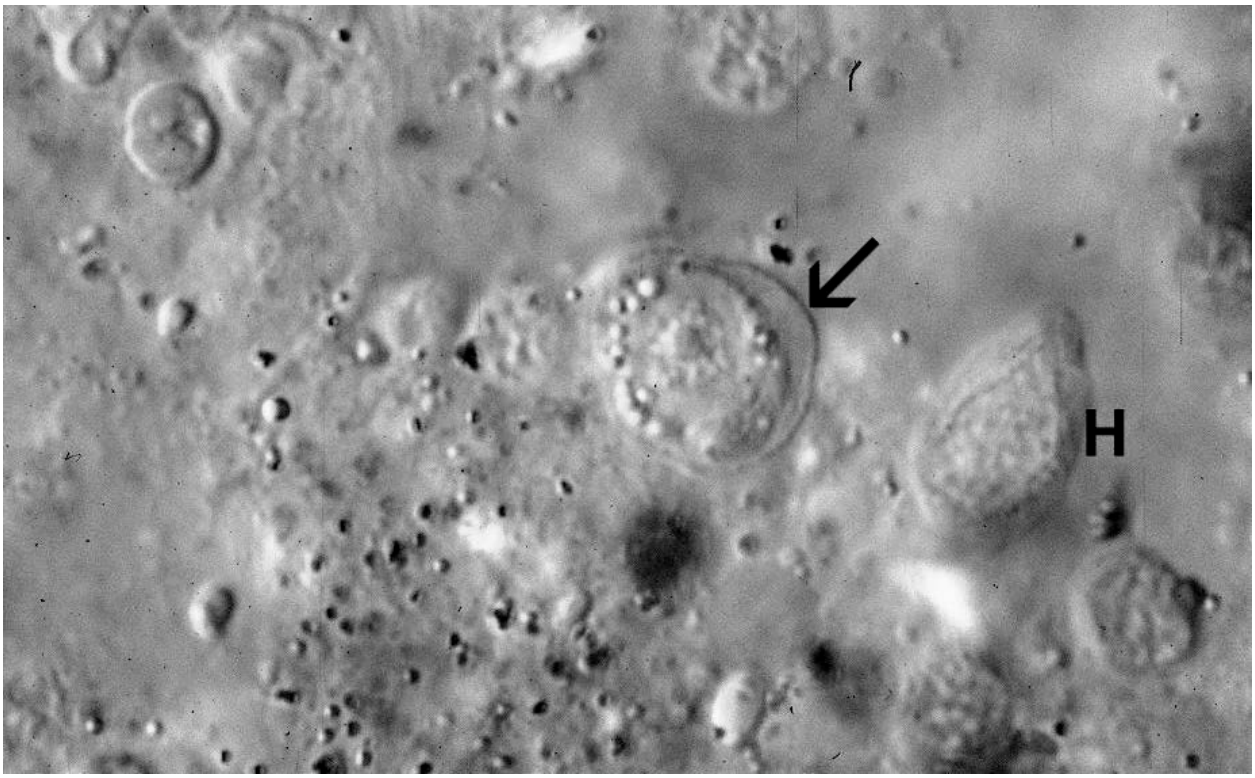


Figure 7. Wet mount preparation of salmonid kidney showing PKX. Note distinctive cell membrane (arrow) and smaller nucleus compared to host cells (H). Cytoplasm of PKX is granular. Nomarski phase contrast.

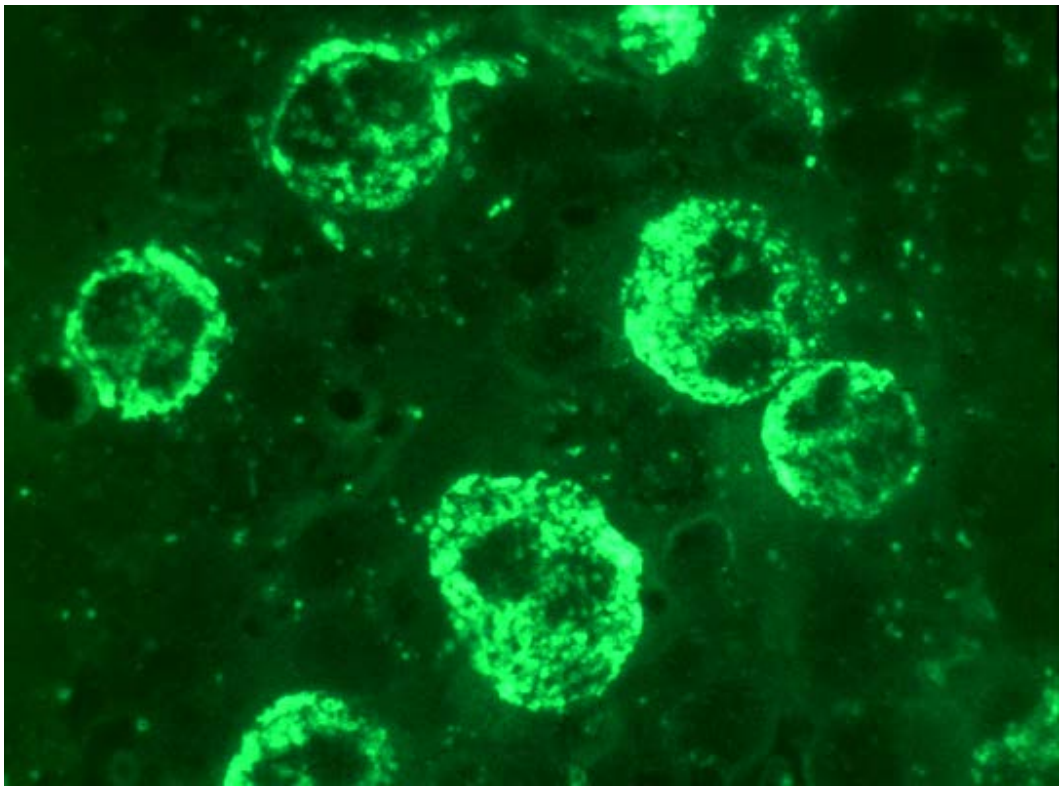


Figure 8. PKX cells in tissue imprints stained with biotinylated GS-1 lectin. Note granular cytoplasm of primary cells.

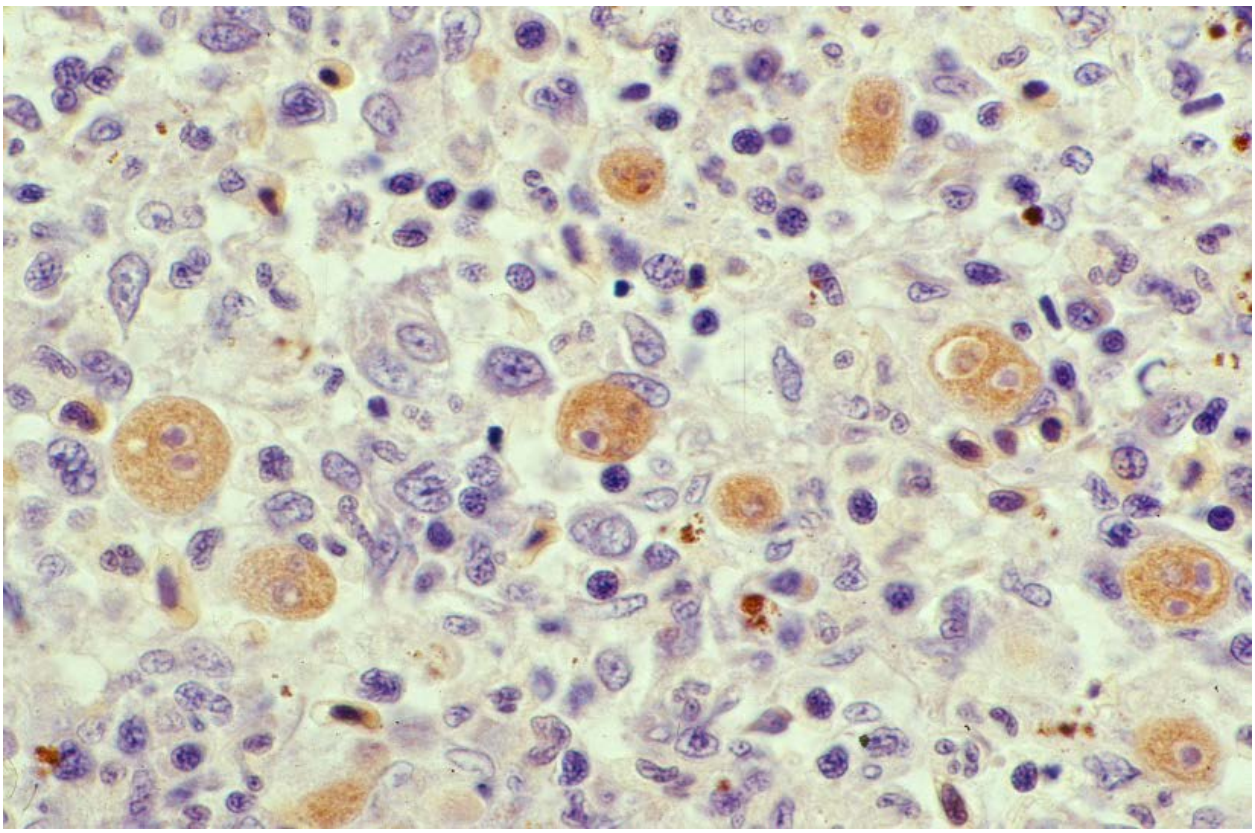


Figure 9. PKX in tissue sections stained with GS-1 lectin.

G. Procedures for Determining Prior Exposure to the Etiological Agent

Sporogonic forms (pseudoplasmodia; Figure 10) are found in the lumina of kidney tubules late in the disease and persist for several months in recovered fish. These forms are comprised of an enveloping cell with many refractile granules and contain a monosporous sporoblast. In wet mounts, the sporoblasts are oblong, 12 X 7 µm and often contain two spherical polar capsules 2 µm in diameter (Kent and Hedrick 1986; Kent et al. 2000). The sporoblast is pliable due to apparent incomplete development of the spore valves. The presence of these sporoblasts in the lumina of kidney tubules during the fall or winter may indicate prior infection by the interstitial form of PKX. The diagnosis of PKX based on the presence of these forms only, however, should be tentative because an inexperienced diagnostician may confuse these incomplete spores with those of other coelozoic (lumen dwelling) myxosporeans that infect the kidney. The PCR tests are useful for confirming the identity of sporogonic forms of PKX (Kent et al. 1998). These PCR tests have also been used to confirm the identity of PKX in bryozoans.

Morris and colleagues (see review by Morris and Adams 2002) have used these PCR primers in *in situ* hybridization tests to identify the parasite in tissue sections. Using this approach, Morris et al. (1999) further linked the intraluminal sporogonic forms to PKX

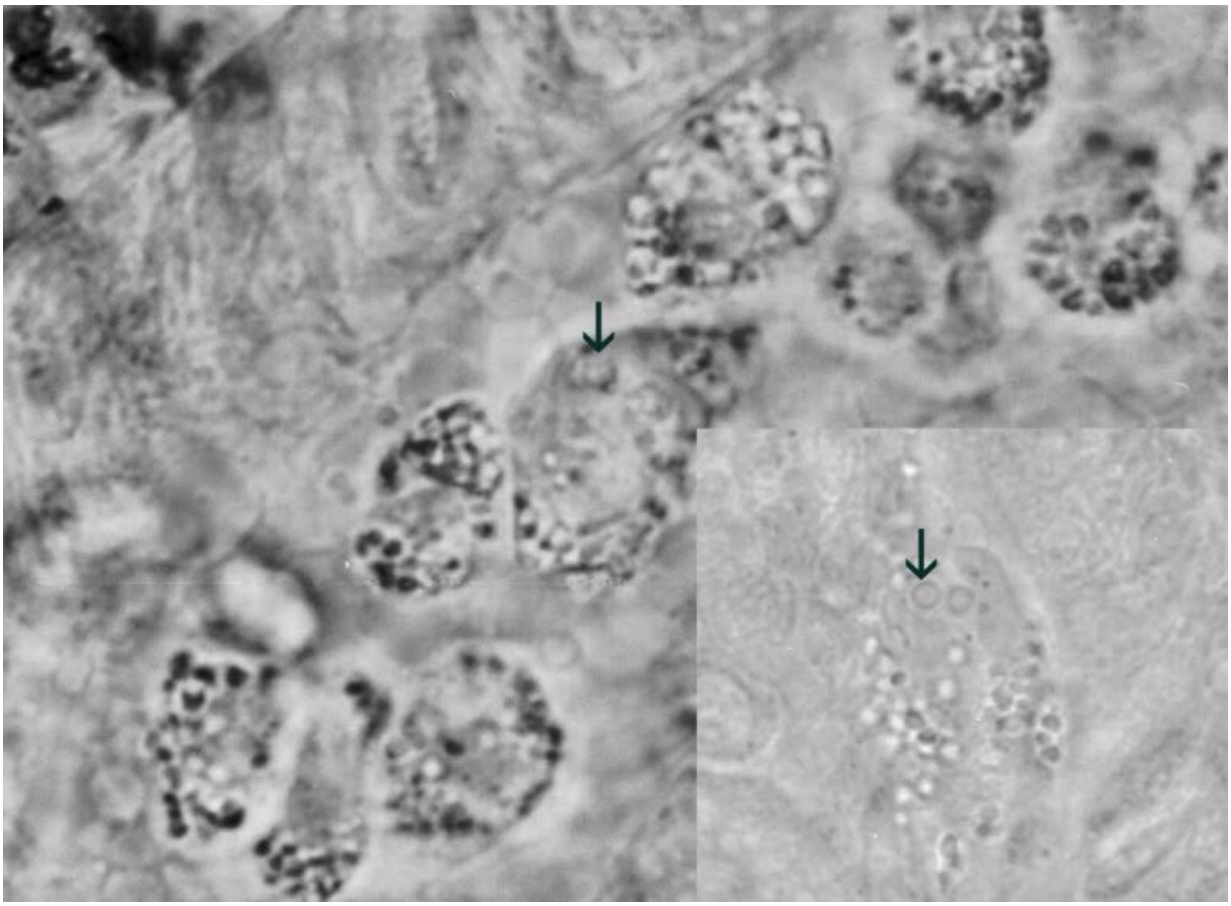


Figure 10. Wet mount of kidney showing PKX sporogonic forms in the lumen of kidney tubules. Arrow = polar capsules.

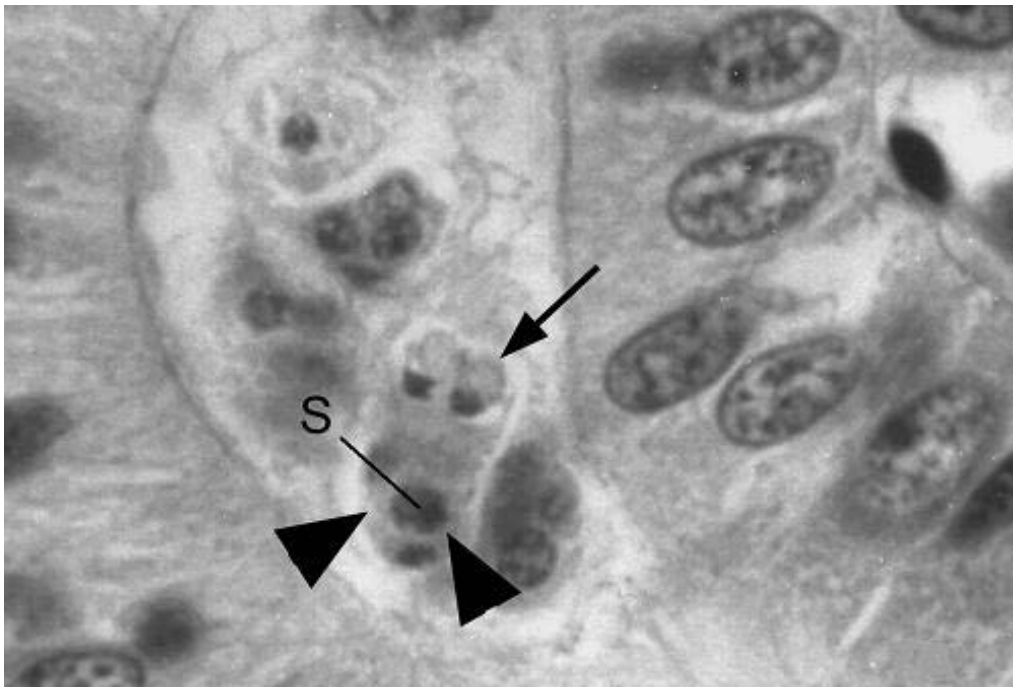


Figure 11. Histological sections of kidney tubules with spores of PKX. Hematoxylin and eosin stain showing polar capsules (arrows), sporogonic nuclei (S).

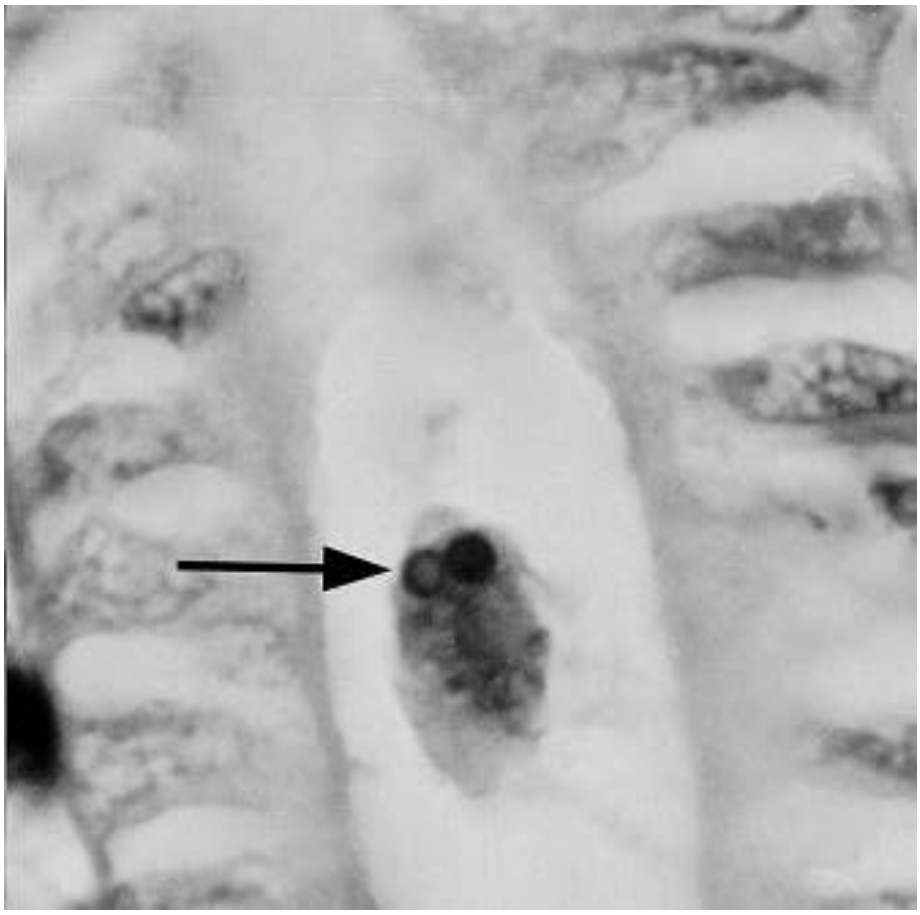


Figure 12. Histological sections of kidney tubules with spores of PKX.). Giemsa stain showing dark blue polar capsules (arrows).

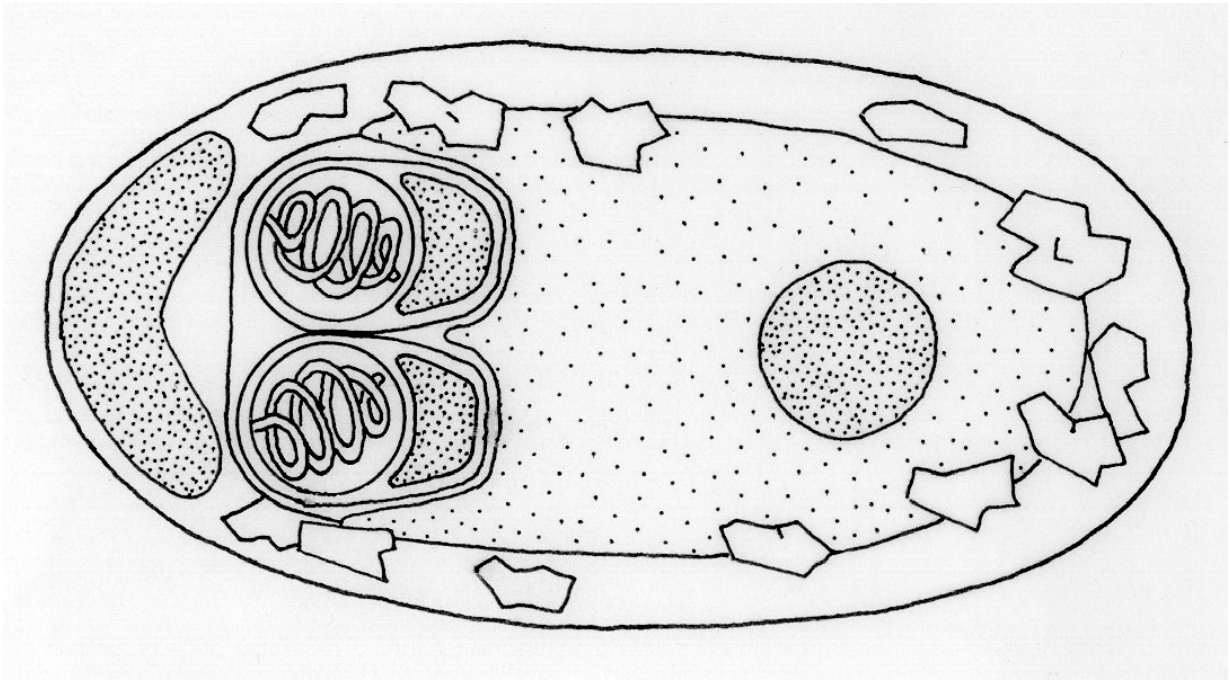


Figure 13. Line drawing of PKX sporoblast within pseudoplasmodium from kidney tubules.

H. Procedures for Transportation and Storage of Samples to Ensure Maximum Viability and Survival of the Etiological Agent

Tissues for histological examination should be collected from freshly killed fish and fixed immediately on site in either Davidson's or Bouin's solution. Imprints should also be prepared from freshly killed fish. The PKX parasite may deteriorate very rapidly in iced or frozen samples.

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