

2.1.2 General Procedures for Electron Microscopy Applications in Diagnostic Virology

Jan Lovy¹ and Dorota W. Wadowska²

¹N.J. Division of Fish & Wildlife, Office of Fish & Wildlife Health & Forensics, 605 Pequest Rd. Oxford, NJ 07863, USA

²Electron Microscopy Laboratory, Atlantic Veterinary College, University of Prince Edward Island, 550 University Ave. Charlottetown, PEI C1A 4P3, Canada

I. Introduction

With the large number of novel and emerging fish viruses, it is important to have methods in place to identify these viruses when they are encountered. Considering that no ready-made immunological or molecular diagnostic tests (e.g. FAT, ELISA, PCR, or serum neutralization) can be applied in these cases, alternative methods need to be employed when dealing with any suspected viral etiology. Transmission electron microscopy (TEM) can be used to directly visualize virions and often identify a virus to family based on ultrastructure.

Novel viruses may be encountered in cell lines during a cell culture assay or a suspected viral etiology can be encountered during histological analysis of organs. This chapter will outline general TEM techniques that can be applied to initially detect and identify suspected viruses. For novel viruses, defining the ultrastructure to understand the family it belongs to could help in developing a protocol to sequence the virus. Considering that TEM protocols vary between laboratories and existing protocols for tissue processing exist, this chapter will focus on when and how these methods can be applied in a diagnostic setting. For detailed protocols of the TEM techniques that are useful in diagnostic pathology refer to the excellent reference by Graham & Orenstein (2007). Lastly this chapter will discuss ultrastructural interpretation including cellular elements in fish cells that can be confused for viruses.

II. When to use TEM techniques

The TEM technique to be applied depends on the sample of interest. In a diagnostic setting, the need to do TEM may arise from two main situations: A) in a viral cell culture assay where a unique pattern of cytopathic effect (CPE) occurs and/or when testing for known viruses has proven inconclusive, or B) when histopathology examination has revealed novel lesions that are suggestive of a virus, i.e. tissue/cell necrosis and the presence of inclusion bodies within cells. In many instances the need to do TEM arises after all tissues have been fixed and processed. The ability to use these processed tissues for TEM to diagnose viruses will be discussed.

In mammals the most common diagnostic samples are body fluids including urine, fecal samples, cerebrospinal fluid, aspirates, blister fluid, tears, and bronchoalveolar lavage fluid to be analyzed by way

2.1.2 Electron Microscopy for Diagnostic Virology - 1

of negative staining (Goldsmith & Miller 2009). With aquatic animals this is less common because of the fast dilution in the aquatic environment and the low concentration of virus in these fluids. Negative staining applications are most frequently used for cell culture studies. Despite this, it is possible to process tissue homogenates, ascites, or intestinal samples for negative staining. In these cases centrifugation methods can be used to help concentrate the virus. Figure 1a is an image of koi herpesvirus from a brain homogenate as seen by negative staining.

A. Investigation of cytopathic effect (CPE) in cell culture

There are a growing number of cell lines from a variety of fish species available to the diagnostician. Generally the selection of the cell line for use is one that is known to be susceptible to the virus of interest or a cell line that closely matches the origin of the tissues that are being tested. Virus infected cell lines produce changes in cell morphology or cytopathic effects (CPE), which may or may not be pathognomonic for several viral agents. To investigate CPE with an unknown etiology there are two recommended TEM methodologies, each with differing advantages, which can be utilized to visualize viral particles. In method-1, the cell culture sample can be processed in a way to directly observe the virions, which are coated onto a TEM specimen grid and the virus visualized by way of negative staining. The advantage of this technique is that it is simple and can yield fast results (same day). A major disadvantage is that the virus has been separated from the host cells and therefore it is not possible to determine the replication location of the virus within the cells and the CPE cannot be well characterized. As previously mentioned, this technique can also be applied to tissue homogenates or fluid samples from the fish. Method-2 provides for a more direct visualization of the virions and allows for description of the CPE, i.e. presence of inclusion bodies, location of replication, and cellular pathology. In this method the infected cells should be fixed during peak viral replication. Although this method takes longer (days), it provides better characterization of the virus. Figure 2 shows an example of infectious salmon anemia virus as seen by both processing methods. Only the outer morphological details can be seen when virus is processed by method-1 (Figure 2a), whereas various replication stages within the cells can be observed using method-2 (Figure 2b,c). Additionally Figure 3 shows an aquareovirus within the TO cell line.

Method-1. Visualization by negative staining

When taking a preparation from cell culture if the CPE is nearing 100% then the growth medium is harvested, whereas if less than 70% CPE is observed then the growth medium and cells should be harvested and lysed with a freeze/thaw cycle. These preparations can either be used directly or they can be spun at low speed (800 G) to remove cellular debris and the supernatant taken for observation by TEM.

Briefly, a 10 µl preparation is taken from the cell culture, placed on a formvar and carbon coated grid, this is followed by the addition of 10 µl of negative stain (e.g. phosphotungstic acid). The solutions are left on the grid for a few seconds to 1 minute followed by drying with filter paper. The grid is then ready to be viewed using a TEM. With this method the background is stained and particles, including intact virions are left unstained, therefore outer details of the virus are visualized against the electron-dense background. Care must be taken with interpretation since the sample contains other cellular debris that can be in the size range of a virus. Notice the cellular debris in the negative staining sample in Figure 1a; careful interpretation must distinguish between virus and other cellular elements, such as broken down

2.1.2 Electron Microscopy for Diagnostic Virology - 1

cellular membranes. Additional information regarding negative staining in diagnostic virology can be found in Goldsmith & Miller (2009).

Method-2. Cell fixation and thin sectioning

This method is recommended for characterizing viral morphogenesis and cellular pathology in cell lines. The suspected virus can either be grown in a monolayer or a suspension culture. The timing of fixation in either case is dependent on how quickly CPE occurs within the cell monolayer. For example, fixation should proceed when a substantial portion of the cells are showing signs of CPE, as it is best to fix cells prior to their death and during peak viral replication. A good rule of thumb is to fix cells when CPE is around 50%, in which case both the growth medium and monolayer should be fixed and processed. This way the sample should contain a combination of healthy cells, cells in various stages of virus infection, and dead/lysed cells. Fixation is accomplished by adding a solution of 6% glutaraldehyde in appropriate buffer to the cell flasks at a volume equal to that of the growth medium. If the CPE is less than 30% of the monolayer, then only the monolayer should be fixed, and in this instance the medium is removed from the flask and 3% buffered glutaraldehyde is added in a sufficient volume to cover the bottom of the flask. After fixation for 1 hour at room temperature (RT), the cells are scraped from the bottom with a soft plastic Pasteur pipette. Following fixation, the cell suspension is spun at 800G for 10 minutes to form a pellet and the cells are washed in buffer. Following washing, the cells are post-fixed in 1% osmium tetroxide and embedded into 2-4% low melting point agar. Once the cells are embedded in agar they can be processed routinely for TEM. Refer to Graham & Orenstein (2007) for additional protocol details.

B. Investigation of histological lesions suggestive of virus

Routine histopathology is an important technique to detect diseases within organ systems and can provide clues to detecting viral infection in fish organs. Unlike cell culture, which is selective for viruses that are susceptible to a particular cell line, histology is non-selective and will detect pathological changes related to viruses. If a viral etiology is suspected based on examination of histological sections, then TEM can be conducted on the sample to link the pathological lesions with the ultrastructural presence of virus. A number of different methods can be successfully used to detect viruses using TEM depending on the starting point of the sample. All of the following samples can be used to detect viruses from tissues, although tissue quality worsens respectively:

- 1) Ideally fixed tissue is fresh tissue preserved in a primary EM fixative (contains a percentage of glutaraldehyde), followed by secondary fixation with 1% osmium tetroxide.
- 2) Fresh tissue fixed in formalin.
- 3) Tissue removed from a paraffin block.
- 4) A portion of a 5 μ m Hematoxylin & Eosin (H&E) stained section lifted from a glass slide.

It is advisable to use TEM-fixed or formalin-fixed tissues if these are available, since these will yield the best results (e.g. Figure 2d). The visualization of virus from tissues removed from paraffin blocks and from routinely prepared histology sections has the advantage of examining archived histological samples, irrespective of age. The use of paraffin blocks and histology slides does, however, limit interpretation of other cell details due to inadequate preservation. Refer to Lovy & Friend (2014) to see a comparison of cell pathology and herpesviral ultrastructure when tissues are processed from formalin vs. taken from

2.1.2 Electron Microscopy for Diagnostic Virology - 1

paraffin blocks. To aid in viral characterization, it is suggested that the specific cell types infected with virus are noted where possible (this may only be possible in glutaraldehyde or formalin-fixed tissue). Understanding the cellular tropism for viruses is helpful in characterizing viruses, for example it was determined that viral hemorrhagic septicemia virus (VHSV) has a tropism for endothelial cells and fibroblasts (Figure 4).

1. Ideally fixed tissue for TEM

If a virus is suspected as the cause of a disease condition then it is advisable to fix matching samples for both routine histology and for TEM processing. Primary electron microscopy fixatives include 2-3% buffered glutaraldehyde or a combination of formaldehyde and glutaraldehyde. Tissues preserved for TEM should be initially fixed for 1 h at room temperature and subsequently kept at 4°C. It is recommended that tissue be further processed within 1 week of initial fixation, although Graham & Orenstein (2007) report that little damage is done to tissue when stored in glutaraldehyde for extended periods (i.e. one year). Secondary fixation is done in 1% osmium tetroxide; tissue must never be stored in osmium tetroxide. Having matching or correlate samples will allow for initial screening of tissues using routine histology and subsequent selection of appropriate specimens for TEM. Plastic embedded semi-thin sections (0.5 µm) should be primarily cut to screen tissues in order to select areas to examine with TEM.

Note that when selecting a buffer to use for fixation, it should match the animal or organ of interest. For example with shellfish it is necessary to use filtered seawater or a buffer that matches the osmolality and pH that the shellfish live in. A good buffer to use for marine shellfish is a sea water mix that can be dissolved in water, such as Instant Ocean (United Pet Group, Blacksburg, VA). If this is not available then filtered seawater can be utilized. Considering that glutaraldehyde does not penetrate deeper than 1 mm it is also necessary to cut tissues into pieces no larger than 2 mm³ when fixing for TEM. Larger pieces can be used in certain tissues that have a large surface area, for example gill tissue.

2. Formalin-fixed tissue

Archived formalin-fixed tissue may be secondarily fixed in 2% glutaraldehyde followed by washing and fixing in 1% osmium tetroxide. This tissue can then be processed following a standard TEM processing protocol. It is important that the tissue is fresh when originally preserved in formalin. It is recommended that tissue is transferred into glutaraldehyde from formalin as soon as possible (Graham & Orenstein 2007). In terms of diagnostic value for virology, tissue fixed this way is often more than adequate to detect the virus and the tissue can yield important information on cell pathology. For example, Figure 1b is a herpesvirus in tissue that was originally fixed in formalin and secondarily fixed in glutaraldehyde.

3. Tissue recovered from a paraffin block

This technique is used when wet-fixed material (formalin or glutaraldehyde) is no longer available for routine processing. Although the tissue quality is compromised, for viral diagnostics this technique is very helpful since in many cases the viruses are well preserved during routine histological processing. A major limitation of this method is that cellular membranes are not preserved therefore ultrastructural interpretation is limited. This may become an issue when analyzing viral morphogenesis because host-derived envelopes may not be visible on the virus. Refer to Lovy & Friend (2014) to see a comparison of tissues processed for TEM from formalin vs. tissues processed from paraffin blocks.

2.1.2 Electron Microscopy for Diagnostic Virology - 1

To apply this technique, the area of interest is examined on a routinely processed H&E slide and that area is marked on the corresponding paraffin block. This is cut out from the paraffin block and deparaffinized by incubating in several changes of xylene to fully eliminate residual paraffin from the sample. Graham & Orenstein (2007) recommend to first place paraffin embedded tissues on filter paper in an oven at 60-70°C to initially melt away the paraffin followed by xylene deparaffinization. Tissues can then be rehydrated through a series of ethanol grades to buffer and the hydrated tissues can be routinely processed for TEM starting with glutaraldehyde fixation.

A second method exists in which osmium tetroxide fixation is done in xylene and glutaraldehyde fixation is skipped. With this method it is not necessary to rehydrate the tissues and instead osmium tetroxide (in crystal form) is freshly prepared in xylene to yield a 1% osmium tetroxide solution in xylene. Tissue is then cleared with the appropriate EM clearant (for example propylene oxide or acetone) and infiltrated/embedded in resin following standard protocols.

4. Histological section lifted from a glass slide

In cases where only a slide is available from a sample or when the lesion of interest is a rare occurrence then this method can be utilized. A routinely processed and stained slide is examined in order to identify the area of interest. This area is marked on the underside of the slide with a diamond pencil. The slide is placed overnight in xylene or acetone solution for removal of the coverslip. Parts of the section that are not of interest are scraped off with a razor blade. A drop of a 1:1 mixture of propylene oxide:Epon is placed over the marked area and allowed to incubate at room temperature for 2 hours. The slide is kept covered to prevent evaporation of propylene oxide. This is followed by incubation in 100% Epon for another 2 hours. To embed the Epon-infiltrated tissue, a flat bottom capsule is filled with 100% Epon and placed over the infiltrated tissue and the slide is placed in the oven at 70°C for 48 hours for polymerization. Following polymerization the capsule is removed by submerging the slide with embedding capsule in liquid nitrogen. The capsule is snapped off the slide with the tissue section attached to the end of the block. This block can be trimmed and ultrathin sections cut for routine TEM analysis. Considering that neither, glutaraldehyde or osmium fixation was done, the tissue preservation and contrast will be reduced in this sample.

III. Ultrastructural Interpretation

Ultrastructural interpretation is a critical component for the correct identification of viruses within a sample. When TEM techniques are applied to diagnostic samples it is often for the purpose of detecting a virus and thus there may be a bias to closely scrutinize the tissue to find a particle that resembles a virion. Generally in cell culture the virus has been amplified to such high concentrations that detecting and identifying the virus should be relatively straightforward. If it is not, then other causes for CPE in the cell line, such as toxicity, should be investigated. In cases when fish tissues are examined the viral concentrations may be lower. Strict criteria must be utilized in order to be confident that a virus is responsible for the associated pathology and caution must be practiced to avoid misidentifying normal cell structures that may resemble a virus. When a viral etiology is suspected and searched for in tissue it is amazing how many cell structures can resemble a virus! It should also be mentioned that although the finding of a virus associated with a lesion provides evidence that a virus is the suspected cause, the lack of finding a virus does not rule out a viral etiology. Although clinically infected animals may have massive amounts of virus in the tissues, in some instances it is possible that a virus infection had run its course

2.1.2 Electron Microscopy for Diagnostic Virology - 1

leaving only the tissue pathology behind, or that the virus had entered a latent state. This may be the case for some of the tumor-associated retroviruses or papilloma viruses. Unless active replication is caught in the tissues, one may only see the proliferative cells with no evidence of virus. For example, a viral etiology was suspected in tank-maintained brook trout with papillomas on the ventral surface, characterized by proliferative epithelial lesions with an inflammatory infiltrate. An exhaustive TEM search through the lesions of several fish only revealed a single cell suspected to be infected with a virus. Even this cell lacked mature virions and contained a virus in the process of replication, making a proper description impossible (Figure 5).

Virus morphology varies based on the family of virus and sometimes the genus; Cheville (2009) and King et al. (2012) are excellent references for virus morphology. Several online references are also available for virus identification including: http://www.virology.net/Big_Virology/BVHomePage.html <http://www.ictvonline.org/codeOfVirusClassification.asp>

Host cellular organelles can fall into the same size range as viruses and can resemble viral structures, although clear differences discern cell organelles and virions. The potential for the structures in question to be related to cellular organelles in either normal or pathological states must be ruled out to avoid misidentification of cellular structures as “virus-like”. Several cellular components in the cytoplasm that may be confused for viruses include primary lysosomes, secretory granules (Figure 6a,b), transport vesicles (Figure 6c), glycogen (Figure 7), and crystalline inclusions. In the nucleus structures such as nuclear pores and perichromatin granules (Figure 8), measuring 30-35 nm, are very common and must not be confused with viruses found in the nucleus, such as herpesvirus and adenoviruses. Nuclear pores are found within the nuclear membranes and when sectioned *en face* the pores are clearly visible. In addition to normal cell structures, pathological processes can lead to unusual cytoplasmic or nuclear inclusions that can resemble viral structures. For some families of viruses, assembly of the virions is associated with inclusion bodies that are composed of viral structural proteins, membranes, and sometimes mature viral particles. The inclusions may be single or arranged in a lattice structure, but should only be considered viral if mature virions are observed, since crystalline inclusions and tubular inclusions can occur within host cells without viral involvement. Figure 9 shows a cell with tubular inclusion bodies in the spleen of an apparently healthy rainbow trout. Though these inclusions are suspicious, no viral particles were apparent. Inclusions such as these can be a result of cellular degeneration. Other pathological processes including aging, toxicity, and misfolded proteins can lead to inclusion bodies involving host cell proteins (Cheville 2009).

In finfish, the cytoplasmic granules of an immune cell type, found in lymphoid tissues can be confused with viral-associated structures. In cyprinid and ictalurid fishes the granules in these cells contain electron dense cores enclosed within a vacuole (Figure 10), which can be confused with herpesviruses. A major distinguishing feature between the immune cell granules and herpesviruses are that the granules have a large size variation, whereas viruses generally have a uniform size range. Additionally, the immune cell granules are never found in the nucleus of the cell and through various section planes it is clear that the immune cell granules form a part of a network of granules mostly oriented around the cell centrosome and are not individual structures as expected for a virus. Similar immune cell types in salmonids form a network of rod-shaped granules containing protein that is in a square-lattice arrangement (Figure 11). These rod-structures and the square lattice material should not be confused with viral associated structures/protein. Further information can be found about the structure of these granules in salmonid (Lovy et al. 2008) and non-salmonid fish (Lovy et al. 2010).

2.1.2 Electron Microscopy for Diagnostic Virology - 1

The examples provided above demonstrate several structures in fish cells that can be mistaken for viruses. Considering that little work has been done in the ultrastructural pathology in fish cells, other structures that may be confused with viruses can be encountered. Caution must be practiced when distinguishing between cellular organelles and viruses. Below are some recommended characteristics to note when ultrastructural descriptions of viruses are being made:

- 1) Detailed morphological description, including shape, presence/absence of envelope, etc., including at least 10, and preferably more, measurements of each replication stage to determine mean and size range of the virus. Measurements should be made at high magnifications for best accuracy; optimally between 40,000 to 80,000X.
- 2) If examining tissue/cells then provide details of morphogenesis and location of viral replication in the cell, i.e. nucleus or cytoplasm. Is the virus associated with any cellular organelles or inclusion bodies or are some stages enclosed within a vacuole or budding from cellular membranes? If possible then the cell type that the virus is found in should be identified to determine cellular tropism.
- 3) If from a histology case, does location of the virus correlate with the pathological lesion? It is important to link the presence of virus with the pathological lesion because it is possible to encounter other nonpathogenic viruses that may be innocent bystanders. Another potential complication is the presence of dual viral infections.
- 4) If from histology or cell culture, note on the incidence rate of the virus in the sample, i.e. how many cells in the lesion are infected. If from cell culture, does the degree or type of CPE observed correlate with the number/location of virions in the sample?

ACKNOWLEDGEMENT

We would like to thank Dr. David Groman for taking the time to edit the content of this section. We would also like to thank everyone that has contributed images which were used to assemble the collection of figures for this section.

2.1.2 Electron Microscopy for Diagnostic Virology - 1

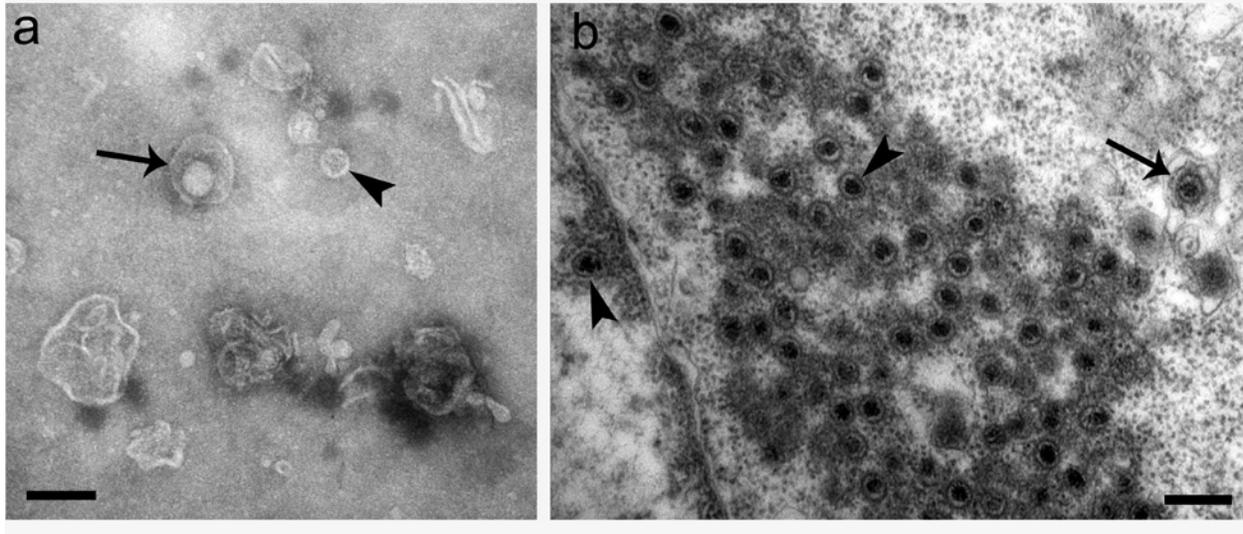


Figure 1. Cyprinid herpesvirus (CyHV), seen using two methods of processing fish tissues. (a) Homogenized brain tissue with CyHV-3 also known as koi herpesvirus, virions observed by way of negative staining. Two forms of the virus include a mature enveloped form (arrow) that resembles a “sunny side up egg” and a naked capsid (arrowhead). Notice the large amount of tissue debris, which can make virus identification difficult. (b) CyHV-2, the cause of herpesviral hematopoietic necrosis, from goldfish kidney tissue initially fixed in formalin and subsequently processed for TEM. Within the cell, viral capsids (arrowheads) occur in the nucleus and cytoplasm. A mature enveloped virion (arrow) is seen in the cytoplasm. Bars, 200 nm. Image (a) provided by Novartis Animal Health, Charlottetown, PEI, Canada, (b) by J. Lovy.

2.1.2 Electron Microscopy for Diagnostic Virology - 1

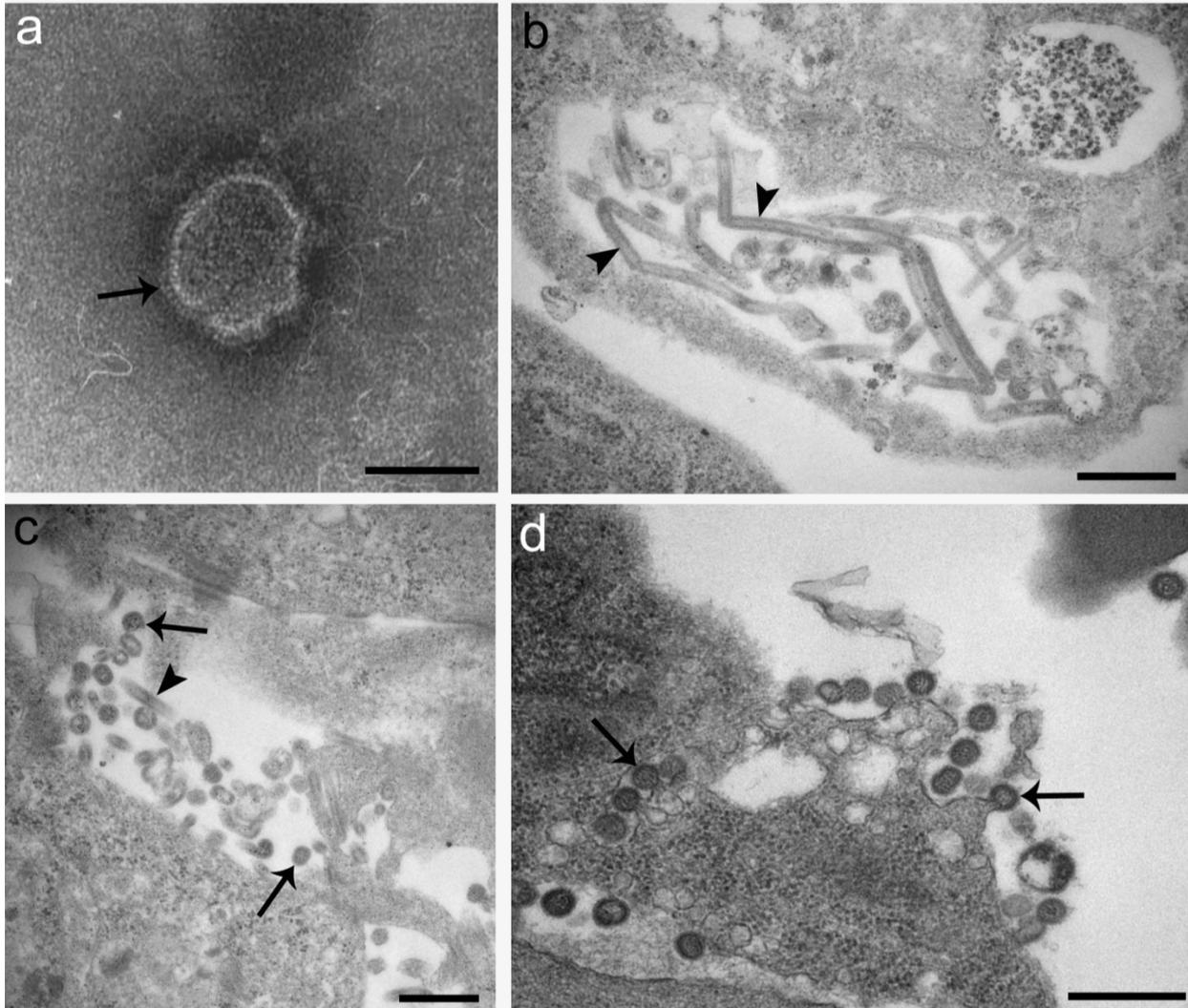


Figure 2. Infectious salmon anemia virus (ISAV) seen by various methods of processing. (a) Processing of an infected CHSE-214 cell line by method-1 in which the virus was purified and negatively stained; notice the surface projections (arrows) typical for the virus. Bar, 100 nm. (b,c) Processing the CHSE-214 cell line by method-2 demonstrating viral morphogenesis within the cells; notice the viral associated tubular structures (arrowheads) and mature virions (arrows) within infected cells. (d) Processing intestinal tissue of an infected Atlantic salmon ideally for TEM; notice the mature virions budding from the plasma membrane of the cell (arrows). Bars (b-d), 500 nm. Images (a-c) provided by Fred Kibenge and image (d) by David Groman.

2.1.2 Electron Microscopy for Diagnostic Virology - 1

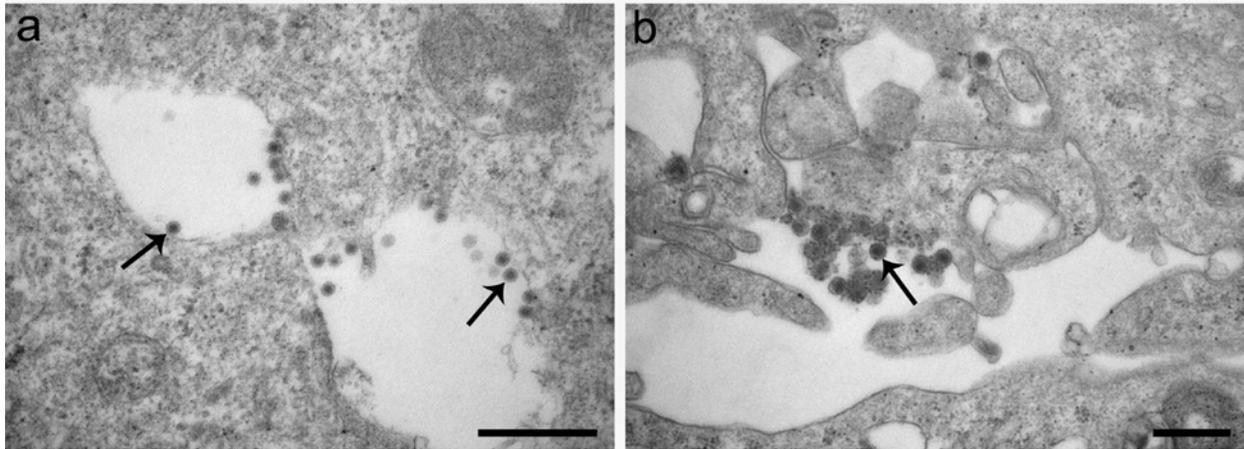


Figure 3. Aquareovirus in the TO cell line processed by method-2 to visualize the virus within the cells; (a,b) virions (arrows) have been shed into the cytoplasm adjacent to the cells. Bars, 500 nm. Notice the resemblance of the virus to secretory granules from normal cells seen in Figure 6a and 6b. Images provided by David Groman.

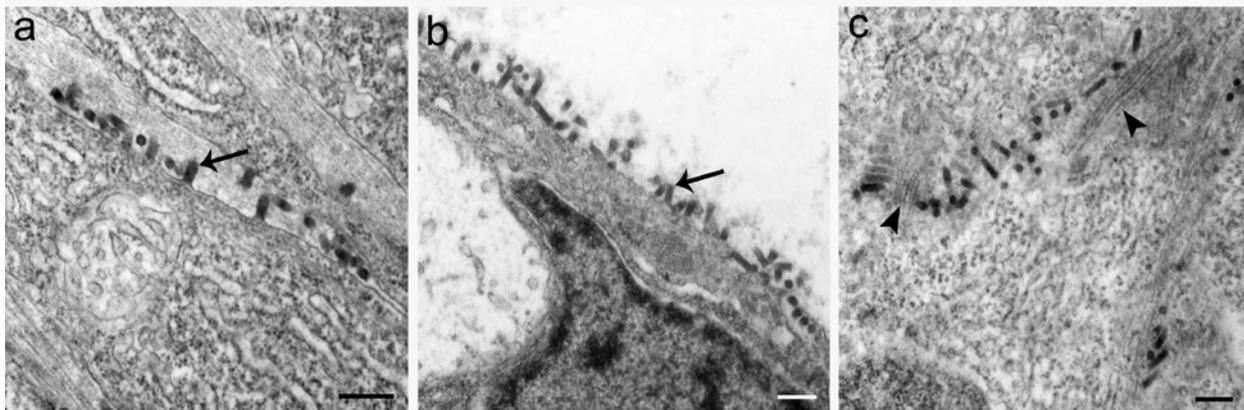


Figure 4. Viral hemorrhagic septicemia virus (VHSV) in Pacific herring tissues ideally fixed for TEM analysis. (a) Virus (arrow) budding from the plasma membrane of a fibroblast and (b) virus (arrow) budding from the surface of an endothelial cell, (c) Filamentous inclusions (arrowheads) are associated with VHSV in the cytoplasm. Bars, 500 nm. Images from J. Lovy .

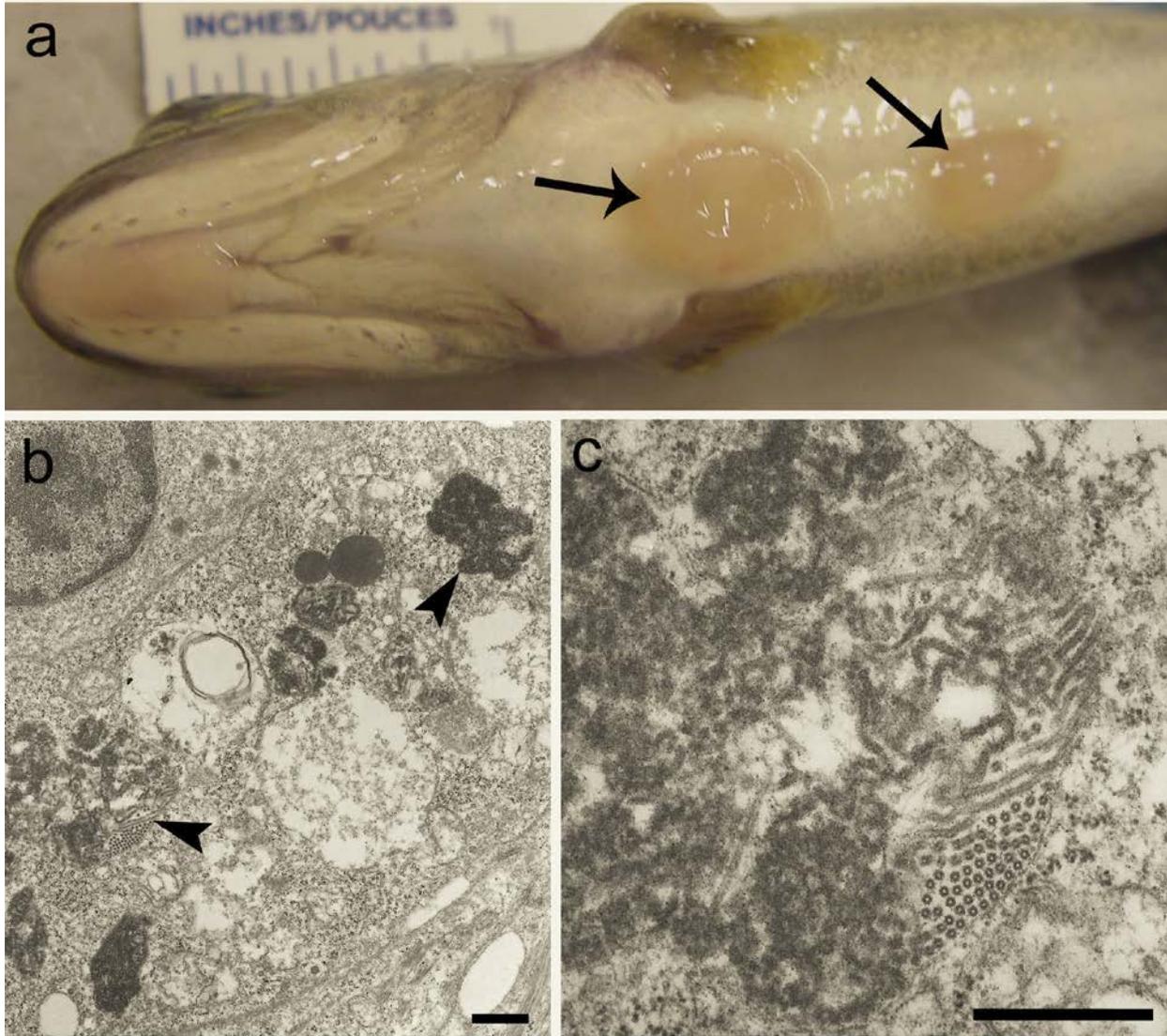


Figure 5. Papilloma in tank-maintained brook trout. (a) papillomas (arrows) were found on the ventral surface of affected fish. Extensive screening of papillomas processed ideally for TEM from several fish revealed only a single cell with viral-associated structures. (b) Electron dense inclusions in the cytoplasm of the cell (arrow), (c) high magnification of inclusion containing viral tubules and 25 nm profiles of nucleocapsids. A proper virus description could not be provided because of the rare occurrence of the virus and only early replicative stages apparent. Bars, 500 nm. Image (a) provided by David Speare, (b,c) from J. Lovy.

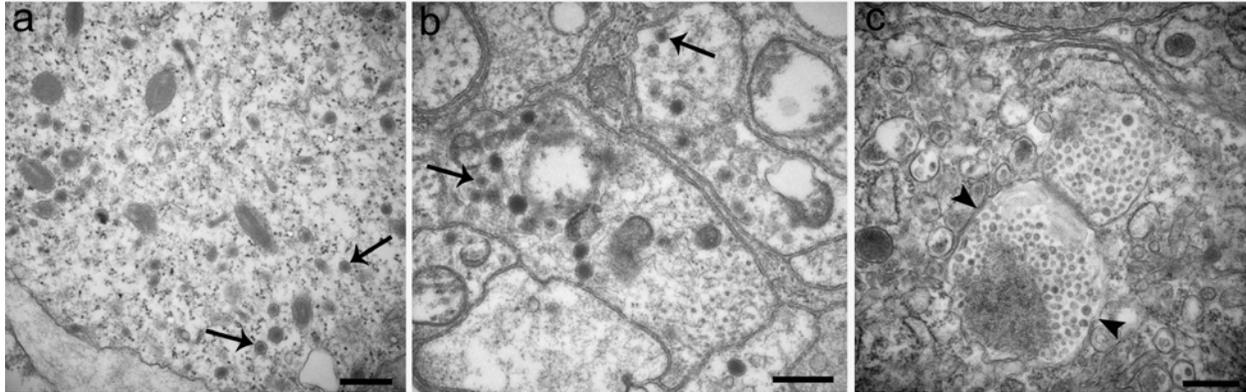


Figure 6. Secretory granules (arrows) and vesicles are a normal component of the cell cytoplasm and should not be confused for viral structures. (a) secretory granules in a channel catfish inflammatory cell, (b) secretory granules (arrows) from an Atlantic sturgeon spleen cell. Notice the resemblance of the secretory granules to aquareovirus in Figure 3. (c) multivesicular bodies (arrowheads) in a hepatocyte of Atlantic salmon. All tissues were fixed and processed ideally for TEM. Bars, 500 nm. Image (a) from J. Lovy and (b,c) provided by David Groman.

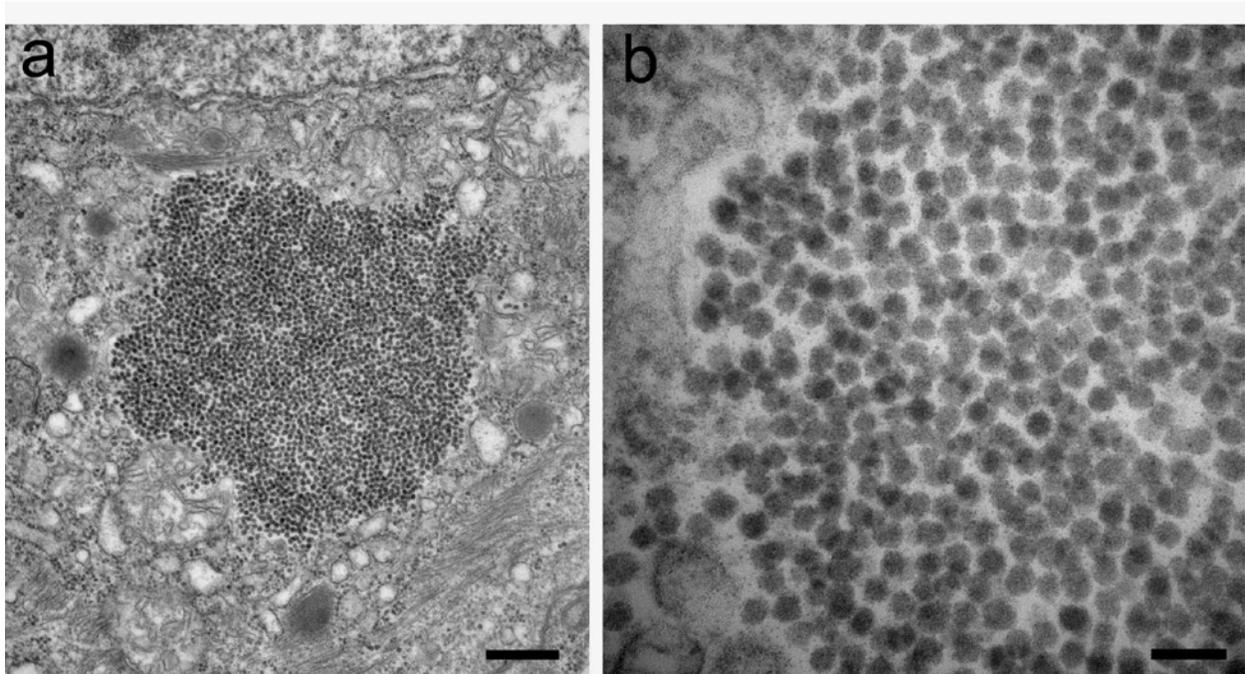


Figure 7. Glycogen in a cardiac cell of Atlantic cod (a) forming a large aggregate in the cytoplasm. Bar, 500 nm. (b) Glycogen is composed of small electron dense structures that measure about 30 nm in diameter. Bar, 100 nm. These structures can be seen individually spread through the cytoplasm or in large aggregates, which can resemble aggregates of virus. Muscle cells and hepatocytes commonly contain glycogen. Tissue ideally fixed and processed for TEM. Images from J. Lovy.

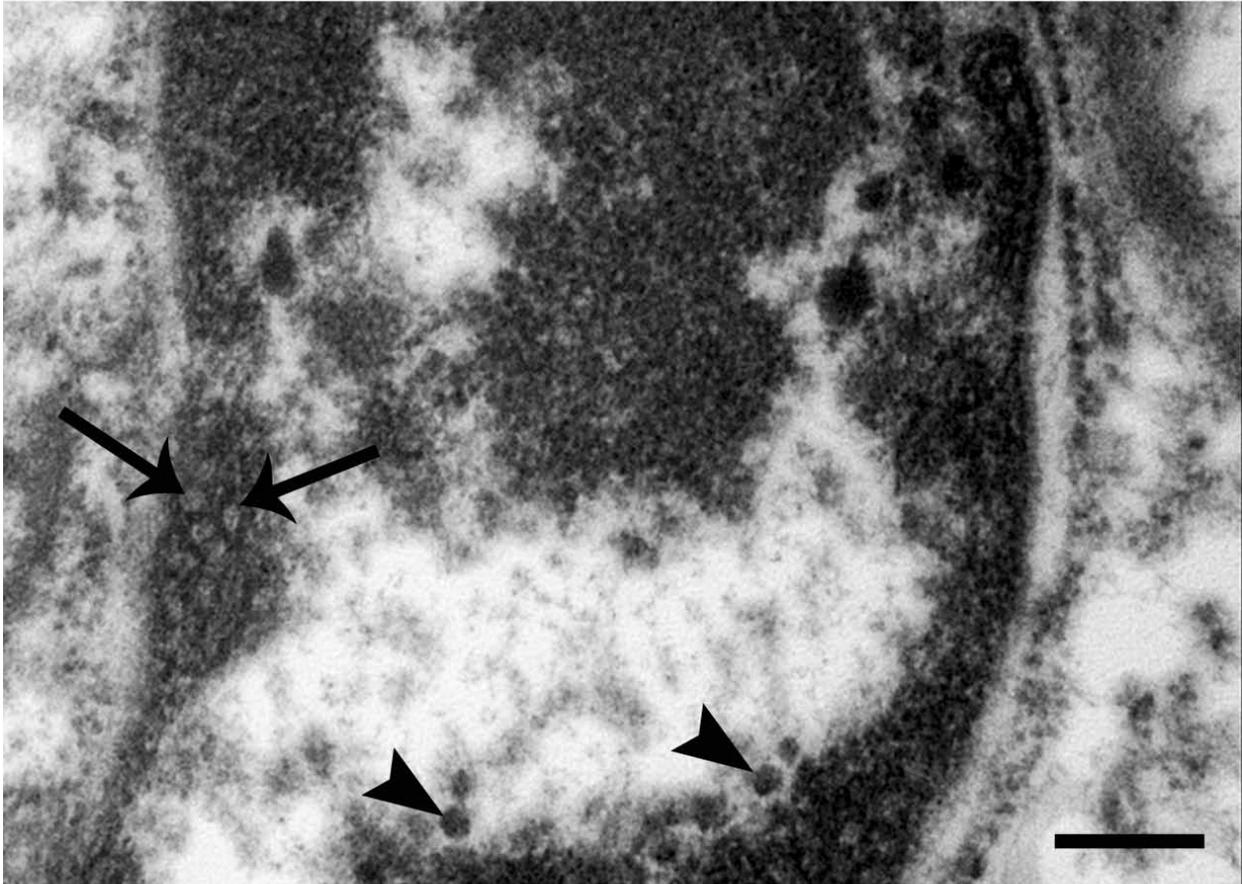


Figure 8. Normal nucleus of a goldfish renal hematopoietic cell containing nuclear pores (arrows) and perichromatin granules (arrowheads). Both structures are found in nuclei of most cells. Bar, 200 nm. Tissue was initially fixed in formalin followed by processing for TEM. Images from J. Lovy.

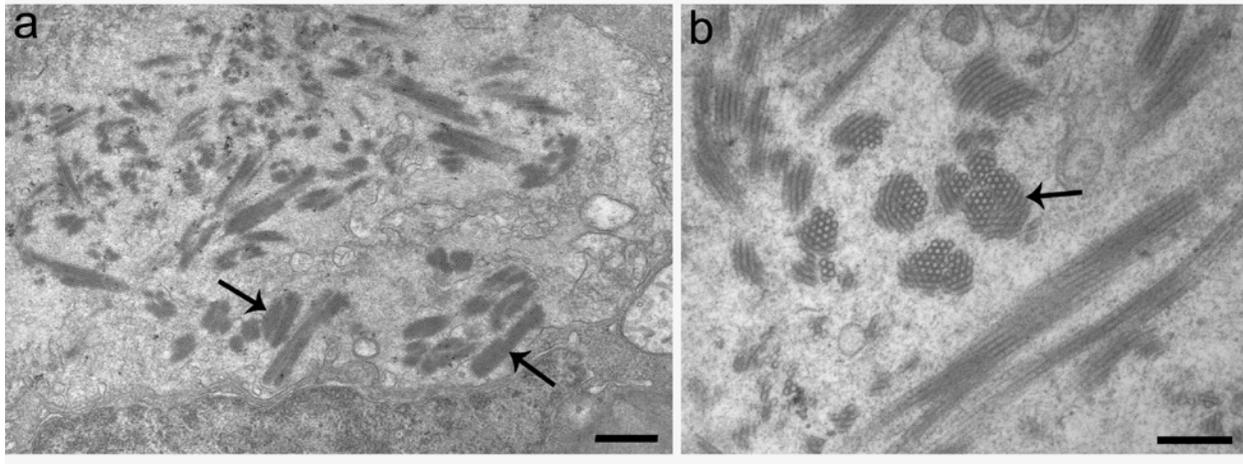


Figure 9. Intracytoplasmic tubular inclusions (arrows) from a splenic cell of an apparently healthy rainbow trout. Inclusions such as these may be associated with a form of cellular degeneration. Bars, (a) 500 nm and (b) 250 nm. Tissue ideally fixed and processed for TEM. Images from J. Lovy.

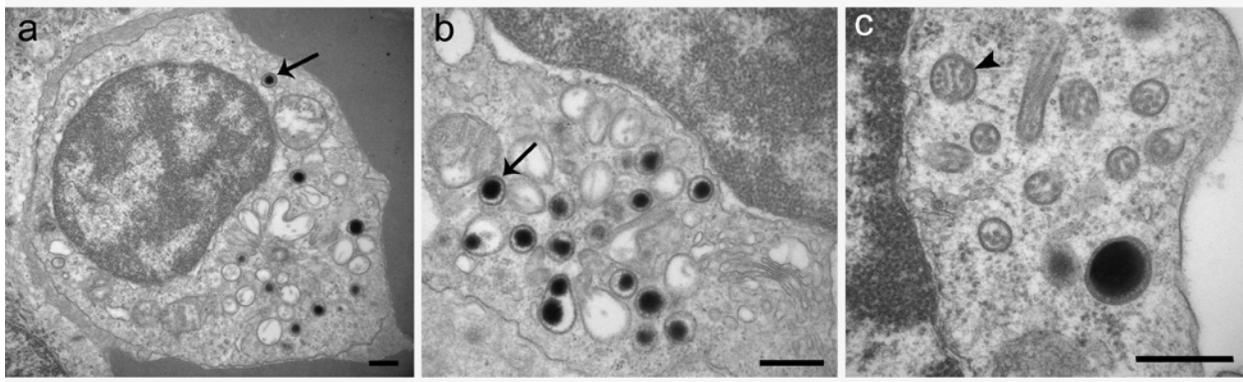


Figure 10. Immune cells in the spleen of cyprinid fish containing a complex network of granules. (a,b) Apparently spherical granules containing a dense core (arrows) should not be confused with herpesviruses. (c) In *Leuciscus idus*, some granules contain material arranged in small vesicles (arrowhead). Bars, 500 nm. Tissue ideally fixed and processed for TEM. Images from J. Lovy.

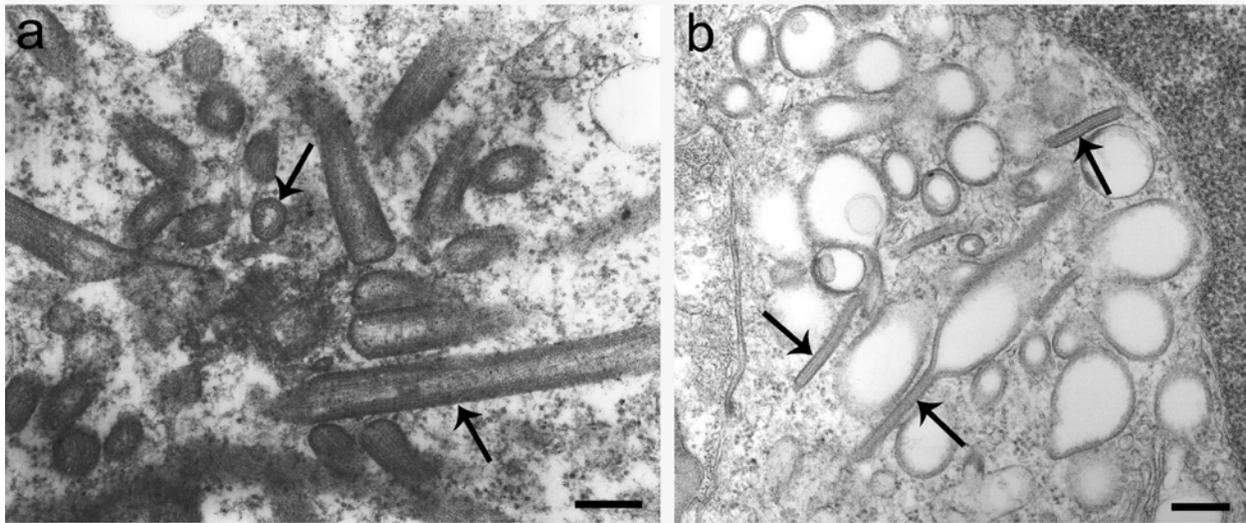


Figure 11. Immune cells in salmonids similar to those from cyprinids (seen in Figure 10). (a) Chinook salmon cell with rod-shaped granules containing a square-lattice structured material (arrows) surrounding a centriole. (b) Vacuolated structures and rod-shaped structures (arrows) in immune cells of Atlantic salmon. Bars, 200 nm. Tissue ideally fixed and processed for TEM. Images from J. Lovy.

REFERENCES

Cheville N. F. and H. Lehmkuhl. 2009. Cytopathology of viral diseases. In *Ultrastructural Pathology*, second edition (N.F. Cheville, ed.), pp.319-423. Wiley-Blackwell, Iowa, USA.

Goldsmith, C. S., and S. E. Miller. 2009. Modern uses of electron microscopy for detection of viruses. *Clinical Microbiology Reviews* 22:552-563.

Graham, L. and J. M. Orenstein. 2007. Processing tissue and cells for transmission electron microscopy in diagnostic pathology and research. *Nature Protocols* 2:2439-2450.

King, A. M. Q., Adams, M. J., Carstens E. B., and E. J. Lefkowitz (eds.) 2012. *Virus Taxonomy*, Ninth Report of the International Committee on Taxonomy of Viruses. Elsevier Academic Press, San Diego, USA.

Lovy, J. and S. E. Friend. 2014. Cyprinid herpesvirus-2 causing mass mortality in goldfish: applying electron microscopy to histological samples for diagnostic virology. *Diseases of Aquatic Organisms*. 108:1-9.

Lovy, J., Wright, G. M., Speare, D. J. 2008. Comparative cellular morphology suggesting the existence of resident dendritic cells within immune organs of salmonids. *The Anatomical Record* 291:456-462.

Lovy, J., Wright, G. M., Speare, D. J., Tysl, T., Dykova, I. 2010. Comparative ultrastructure of Langerhans-like cells in spleens of ray-finned fishes (Actinopterygii). *Journal of Morphology* 271:1229-1239.