

**Poultry, Fisheries &** Wildlife Sciences

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## Enhancing Histologic Assessment of Experimental Disease Using Histomorphometrics

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Histopathology is a valuable tool for assessing disease establishment and temporal lesion progression and as such, is regarded as one of the gold standards for validating experimental disease models. While specific mechanisms of disease often involve complex molecular pathways, subjective scoring of lesions at various experimental times can provide insight into critical points of infection/disease progression, infectious dose, and host response. This often allows the investigator to focus on a single time point to elucidate mechanisms that may be instrumental in understanding disease to institute appropriate management and prevention programs. However, this scientific paradigm is not without an important caveat: evaluation of histologic lesions is a subjective practice and may present as a difference of opinion among pathologists. This is especially relevant when considering subtle tissue alterations and experienced pathologists that are capable of recognizing those changes. Histomorphometry is the quantitative study of microscopic features and substantially reduces subjectivity and observer variation. Histomorphometrics provides a continuum of measurements increasing the statistical inference of experimental design. The recent advances in digital microscopic imagery and computer assisted analysis software have catapulted histomorphometricsas an integral component of pathologic lesion assessment. Morphometric software is readily available and with high quality digital imagery, data sets are reproducible and comparable between individual pathologists. I employ the use of Image J software (download available at: http://rsb.info.nih.gov/ij/) in my laboratory for a wide gamut of diseases, most often studies involving significant respiratory pathogens of poultry. In our studies investigating infectious bronchitis virus (IBV) serotypes, the Image J linear measurement tool was used to readily determine tracheal mucosal thickness (i.e. reflecting the degree of mucosal damage/ulceration and lymphocytic infiltration). While subjective lesion scoring was not always deemed significant in these studies, histomorphometric correlations were significantly associated with clinical signs, viral load and differences between IBV serotypes were easily discerned. Image J methodologies have also been adapted in my laboratory to determine myofiber cross sectional area and interfascicular edema associated with ischemic myopathy in high yield broiler chickens, and in other studies to quantify hepatic lipidosis in experimental aflatoxicosis. We have also found and continue to find additional applications for histomorphometrics, in both experimental and clinical disease. For example, special histochemical staining and immunohistochemistry colorimetric analysis has been used as an adjunct for evaluation of core bone marrow samples in myelodysplasticsyndromes of companion animals. While histomorphometry applications appear limitless, two basic parameters are required to take full advantage of the possibilities: quality sample collection and processing, and histologic evaluation by a skilled pathologist.

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Received March 16, 2013; Accepted March 18, 2013; Published March 19, 2013

Citation: Joiner KS (2013) Enhancing Histologic Assessment of Experimental Disease Using Histomorphometrics. Poult Fish Wildl Sci 1: e101. doi:10.4172/2375-446X.1000e101

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