

# Morphometric Evaluation of Proliferative Lesions in *In-Situ* Fixed Chicken Lungs

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## INTRODUCTION:

ImageJ, a powerful open-source software tool for morphometry, was developed at the National Institutes of Health (NIH) and is available for download from an NIH website ([rsb.info.nih.gov/ij/download.html](http://rsb.info.nih.gov/ij/download.html)) at no cost. Measurements made using ImageJ on H&E stained histologic sections prepared from *in-situ* fixed lungs from broiler breeder and SPAFAS chickens included perimeter of parabronchi and mean gray values. Objectives were to determine if these measurements could be used to differentiate images having lesions from images having no lesions. This use of ImageJ would provide an objective evaluation for comparing lungs obtained from chickens with natural and experimentally-induced diseases. A method for objective measurement of lung responses would be useful in evaluating vaccine and other control programs.

## MATERIALS & METHODS:

H&E stained histologic sections from *in-situ* fixed lungs from 23 broiler breeders, 27-29 weeks of age, 2 SPAFAS chickens, 9 weeks of age, 1 SPAFAS chicken, 6 weeks of age with Marek's tumors in the lung, and 3 broiler breeders, 9 weeks of age were evaluated by taking digital images from at least 25 randomly selected fields using the 2X and 10X microscope objectives. This produced images with on-screen magnification of 68X and 340X. The outer boundaries of the region occupied by each section were determined using the microscope stage scale and pairs of random numbers corresponding to the vertical and horizontal scale values within these boundary limits were generated using an Excel™ spreadsheet. Each image selected by using the random number pairs was scored for absence (1) or presence (2) of lesions.

Perimeter measurements were made on parabronchi from images at the 68X on-screen magnification. Images were converted to 8-bit, edges found, threshold adjusted, and perimeter of parabronchi outlined using the magic wand tool. The measure command in ImageJ generated the perimeter measure.

ImageJ was used to measure the value (0 = black to 255 = white) for each pixel in each image (or selected area) and to calculate a mean gray value. The 340X on-screen magnification was used for mean gray measurements. Point-counting of 219 images from 7 broiler breeders was done at 340X using a grid with 81 points imposed on the image.

Broiler breeders 27-29 weeks old came from commercial flocks under study for suspected calcium tetany. SPAFAS chickens were housed in isolator units and the 9 week-old broiler breeder chickens were housed in floor pens. Marek's tumors were induced by contact challenge with a very virulent Marek's disease virus (vMDV - 648A).

Data were analyzed using a commercially available software program (SPSS, [www.SPSS.com](http://www.SPSS.com)).

## RESULTS:

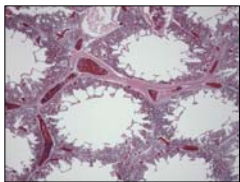


Figure 1. Normal Lung (68X). Broiler Breeder. See Fig 2 for results of adjustments for perimeter measurements.

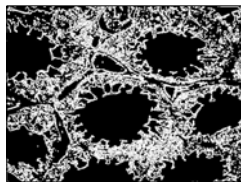


Fig. 2 Normal Lung (68X) - 8-Bit and Threshold Adjusted for Automated Perimeter Measurement

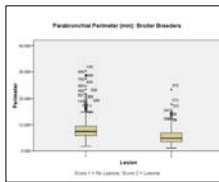


Fig. 3 Box plot comparing median perimeter (mm) of 466 normal parabronchi (median = 7.44mm) with median of 294 parabronchi with lesions (median = 4.83mm). Presence of lesions results in a lower median value. Broiler breeders were 27-29 weeks-old.

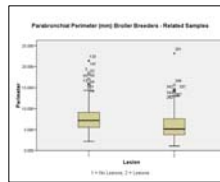


Fig. 4 Box plot of perimeter for 186 parabronchi from 27-29 week-old broiler breeders with no lesions (median = 7.17mm) compared with 186 parabronchi with lesions (median = 5.13mm). Samples are related because they are from the same lung samples. Median is significantly lower (Friedman's nonparametric test asymp. sig. = .000) in parabronchi with lesions.

Parabronchi with lesions had a decrease in median perimeter compared with parabronchi with no lesions. Data did not have a normal distribution so median values using box plots are used for comparisons. Box plots show minimum, first quartile, median (solid line), third quartile, and maximum values. Outliers are identified by data entry number.

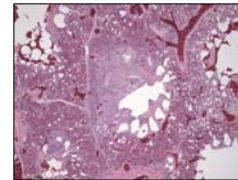


Fig. 5. Parabronchus with lesions (score 2). Broiler Breeder (68X).

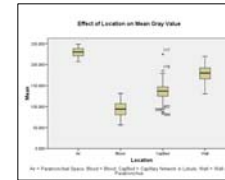


Fig. 6. Mean gray values are influenced by location within lungs. Gray values of each pixel range from 0 (black) to 255 (white). Space or air has the highest value (229) and blood the lowest (94). Values were determined by measuring mean gray value in a standard (0.1mm<sup>2</sup>) selection square.

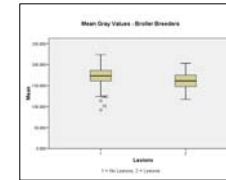


Fig. 7. Mean gray values are lower in lung sections having lesions. 515 images had no lesions (median = 173) and are compared to 258 images with lesions (median = 161). Broiler breeders were 27-29 weeks-old.

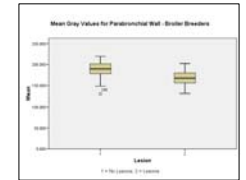


Fig. 8. Mean gray values are lower in parabronchial walls having lesions. (median = 170) than in walls with no lesions (median = 194). A standard square selection area (0.1mm<sup>2</sup>) was used to measure parabronchial walls. 49 images with no lesions are compared with 49 having lesions.

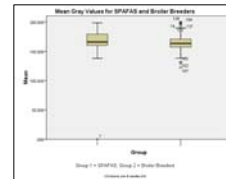


Fig. 9. Mean gray values were similar (median = 166) in 57 images of lungs from SPAFAS controls raised in isolators when compared to 109 images from broiler breeder chickens raised in floor pens (median = 164).

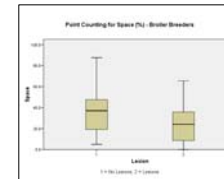


Fig. 10. Point counting shows a decrease in median percent space in lungs of 27-29 week-old broiler breeders with lesions (37% space in 140 images with no lesions; 26% in 79 images with lesions). This observation is consistent with the mean gray value results

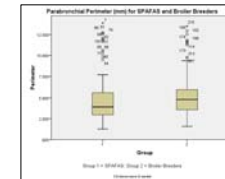


Fig. 11. SPAFAS chickens housed in isolators (controls) had lower median parabronchial perimeter (median of 110 images = 3.9mm) than 9 week-old broiler breeder chickens housed in floor pens (median of 136 images = 4.8mm). Median test asymp.sig. = .007.

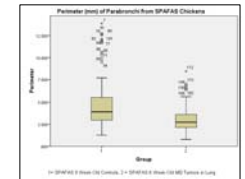


Fig. 12. Median perimeter of parabronchi are lower (median of 82 images = 2.7mm) in a SPAFAS chicken with MD tumors in the lung than median perimeter of parabronchi in SPAFAS controls (median of 110 images = 3.9mm).

## SUMMARY AND CONCLUSIONS:

Measurements of parabronchial perimeter were made easily using ImageJ and proved useful as these measurements were lower in parabronchi with lesions. Proliferative lesions include hypertrophy and hyperplasia of respiratory epithelium lining parabronchi including the atria and infundibulae. Hypertrophy of smooth muscle is a prominent feature. These changes are expected to decrease parabronchial perimeter and results of our study confirm this. Proliferative lesions are expected to decrease the relative amount of space (air) as cells increase in size and number. This pattern is expected to cause mean gray values to decrease. This effect is confirmed by the results presented here.

These results support the concept of using ImageJ to generate objective data to aid in the differentiation of lungs with lesions from those without lesions.

