ASSESSMENT OF SELECTION AND MISCLASSIFICATION BIASES IN MOLECULAR EPIDEMIOLOGIC STUDIES

Singer RS[1], Carpenter TE[2], Jeffrey JS[3], Cooke CL[4], Hirsh DC[4]

[1]Department of Veterinary Pathobiology, University of Illinois, Urbana, IL, USA, 61802
[2]Department of Medicine and Epidemiology, University of California, Davis, CA, USA, 95616
[3]Department of Population Health and Reproduction, University of California, Davis, CA, USA, 95616
[4]Department of Pathology, Microbiology and Immunology, University of California, Davis, CA, USA, 95616

In infectious disease epidemiologic studies, researchers often rely on specific cues of the host, such as clinical signs, as surrogate indicators of pathogen presence. A selection bias would manifest if the specific visual cues used in sampling for the pathogen were not representative of the full range of signs caused by the strains of that pathogen. In addition, in many molecular epidemiologic studies, isolates are collected over extended periods of time. Inferences are then made about isolates collected during this time interval. If a clone of an organism were to be sampled at the beginning and end of this time interval, random genetic drift may result in different DNA fingerprints between the isolates. Consequently, a misclassification bias due to random genetic drift would cause the isolates to be considered different even though they were derived from the same clone. We assessed for the presence of these biases during a one-year molecular epidemiologic investigation of *Escherichia coli* associated with avian cellulitis in broiler chickens. This condition is characterized by a diffuse inflammatory reaction in the subcutaneous tissue that results in the complete or partial condemnation of the carcass at processing.[1,4,5] Numerous investigators have causally linked the presence of *Escherichia coli* with cellulitis.[2,3,6,8] The lesions induced by these *E. coli* can vary considerably in their morphological appearance [1,7] as well as overlying skin involvement.

In our studies, carcasses were collected at the processing plant prior to evisceration so that the skin of the bird was still intact and the lesion was not contaminated. We used visual cues, such as skin discoloration, to select birds that we suspected to have lesions, and this method may have resulted in a selection bias. Therefore, we utilized a validation protocol to assess the potential for selection bias in our molecular epidemiologic studies of *E. coli* and avian cellulitis. In two different trials, *E. coli* DNA fingerprints were compared between birds that our observers collected and the birds that the observers missed. Using Fisher's exact tests and simulation models, we determined that the isolates collected by the observers were not significantly different

from the isolates missed by the observers ($P > 0.60$ in both trials). Our method of selecting birds suspected of having cellulitis did not significantly bias our inferences about the population of *E. coli* associated with cellulitis in the flock.

Because the carcasses were collected over a one-year period, we also evaluated the potential influence of misclassification bias due to random genetic drift. We designed an experiment in which antibiotic resistant mutants of four broiler-derived isolates of *E. coli* were inoculated onto the litter of an artificial broiler environment. Forty 14-day-old broilers were living on the litter when the isolates were inoculated. Litter was sampled by drag swab weekly for five weeks, and a minimum of 24 colonies of each antibiotic-resistant isolate were DNA fingerprinted using pulsed-field gel electrophoresis at each sampling interval. We observed no genetic drift during this study. Consequently, the genetic structure of *E. coli* isolates in the broiler chicken environment is likely to be stable over time allowing isolates to be collected reliably over extended periods.

References

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