PERFORMANCE ASSESSMENT OF DIFFERENT DIAGNOSTIC ASSAYS TO IDENTIFY EPM-AFFECTED HORSES IN A CLINICAL SETTING

Rachel Lemcke[1], Rodney Belgrave[1], Jennifer Morrow[2], Nicola Pusterla[3]

[1]Mid-Atlantic Equine Medical Center, Ringoes, NJ 08551
[2]Equine Diagnostic Solutions, Lexington, KY 40511
[3]School of Veterinary Medicine, University of California, Davis, CA 95616.

Current antemortem EPM diagnostic strategies determine serum, CSF, and serum: CSF antibody titers to differentiate acute or heightened infection from general exposure to *S. neurona*. To more effectively identify EPM-affected patients and better evaluate assay performance, we compared paired results from two different EPM assays (the IFAT and the SAG 2, 4/3 ELISA) within a subpopulation of patients at an equine clinical hospital. Sampled across four years, IFAT CSF samples, in addition to paired SAG serum and CSF, were submitted soon after collection (n=88 horses). For a subpopulation (n=18), antibody ratios for serum: CSF for both IFAT and SAG were calculated on paired samples and tested using the same aliquots. EPM-positive samples were defined as: ≥1:160 serum and ≥1:5 CSF antibodies on IFAT; ≥1:250 serum and ≥1:2.5 CSF antibodies on SAG; ≤64 and <100 ratios on serum: CSF ratio for IFAT and SAG assays, respectively. Descriptive statistical analysis, including overall percent agreement (OPA), positive percent agreement (PPA), negative percent agreement (NPA), Cohen's kappa coefficient (k), and McNemar p test, was performed to compare assay components, as a perfect reference EPM test standard is nonexistent. When paired results for the SAG serum: CSF ratio were compared to the IFAT CSF, the OPA, PPA, and NPA were 87.50, 47.37, and 98.55, respectively, indicating that both tests can equally diagnostically exclude EPM ($p<0.02$; n=88). Additionally, the SAG ratio was interpreted as EPM less frequently than the IFAT CSF; moderate agreement between the assays (k=0.55, 95% CI 0.33-0.78) also points to this disparity in EPM identification. Similarly, comparison between the SAG to IFAT serum: CSF ratio results revealed an OPA, PPA, and NPA of 88.89, 85.71, and 90.91, respectively, with stronger agreement between the tests (k=0.77, 95% CI 0.46-1.00**).** These assay results did not demonstrate an identification bias toward either assay (n=18; p=0.48), suggesting the assays could be potentially substituted for each other, though a larger sample size should be considered in future analysis. Comparisons between IFAT or SAG serum versus the SAG or IFAT ratio, respectively, showed a poor agreement of results: OPA and k were 44.44 and -0.23 (95% CI -0.63-0.17), and 44.44 and 0.07 (95% CI, -0.07-0.22), respectively. Additionally, while ratio calculations for both assays require positive CSF samples, positive SAG CSF samples occasionally yielded negative ratios (NPA 60.76; k=0.24 (95% CI 0.10-0.38); n=88). Interestingly, the IFAT CSF perfectly correlated with the ratio (OPA 100; k=1.00; n=18). A lack of corresponding necropsy results on sampled horses, and a small subpopulation for direct ratio comparisons, unfortunately limits assessment of EPM assay accuracy (i.e. sensitivity and specificity), though past research has evaluated assay accuracy (Johnson et al., 2013). Clinicians relying solely on serum testing for a diagnosis may likely mistake infection for exposure given the high seroprevalence (Witonsky, 2016). CSF testing alone seemed appropriate for the IFAT assay, but did not seem sufficient on the SAG assay. In conclusion, clinicians selecting the IFAT assay could potentially only analyze CSF, while the SAG assay seems to perform best when using the serum: CSF ratio.

Johnson, A.L., Morrow, J.K., Sweeney, R.W., 2013. Brief Communication 596–599.

Witonsky, S., 2016. ACVIM Consensus Statement 491–502. doi:10.1111/jvim.13834