The Kenya Veterinarian 18(2) (1994) 270-272

- 6.6 -

RISK FACTORS FOR SEROPOSITIVITY TO EQUINE MONOCYTIC ERHLICHIOSIS: EVIDENCE FOR THE ROUTE OF TRANSMISSION

ATWILL, E.A.[a], MOHAMMED, H.O.[a], LOPEZ, J.W.[b], DUBOVI, E.J.[b]

Equine monocytic erhlichiosis (EME) was first reported in 1979 near the Potomac River in Maryland (Knowles et al., 1983). EME is a blood-borne rickettsial disease caused by *Ehrlichia risticii* (Holland et al., 1985; Rikihisa et al., 1985). Clinical symptoms associated with EME can include severe diarrhea, fever, anemia, leukopenia, ventral and limb edema, abortion, colic, and laminitis (Ziemer et al., 1987). The seasonal nature of seropositivity (Goetz et al., 1986) and EME (Perry et al., 1986), lack of a point source for infection (Perry et al., 1986), and the fact that other ehrlichial diseases are passed by hematophagous insects have lead investigators to suggest arthropod transmission. However, attempts have failed to demonstrate experimental transmission by arthropods (Schmidtman et al., 1986; Hanh et al., 1990).

Recent studies have shown that major antigens and the 16*S* rRNA sequence are more similar between *E risticii*, *E sennetsu*, and *Neorickettsia helminthoeca* than between *E risticii* and the other *Ehrlichia* species (Rikihisa, 1991; Pretzman et al., 1992). Considering that oral transmission to ponies has been demonstrated (Palmer et al., 1988), it is plausible that *E risticii* could be transmitted to horses by helminths or by some other orally-mediated vector (Palmer et al., 1988).

A cross-sectional study was designed to locate counties within New York state with high seroprevalences among the equine population, to determine which host, management, and environmental factors were associated with seropositivity to *E risticii*, and to determine if there was evidence for arthropod or helminth-mediated transmission of *E risticii* into horses.

MATERIALS AND METHODS

Five hundred and eleven equine operations containing 2587 horses were randomly selected from a list frame of 39,000 equine operations comprising the New York state 1988 equine census. These farms were enlisted through voluntary participation.

Blood samples were obtained through a farm visit and sera were stored at -70 C until needed. The procedures used for preparation of antigen and the IFA assay for serum antibodies were adapted from Ristic et al., 1986. Blood samples were classified as seropositive when the IFA titer was greater than or equal to 40.

Information on each horse and on the farm's management practices and surrounding ecology was obtained by personal interview and resource maps. The association between each factor and seropositivity to *E risticii* was tested using random effects logistic regression (Egret, 1990), with a level of significance set at 0.10. Statistical analyses were performed on three strata of animals, the first being all horses enrolled in the study (n=2587; mean residency=5.9 years), the second being restricted to horses born on the property or that had resided at least 4 years on the farm (n=1587, mean residency=7.9 years), and the third strata being restricted to horses born on the property or arriving within 6 months of birth (n=694, mean residency=6.9 years).

[a]Section of Epidemiology, depratment of Clinical Sciences, College of Veterinary Medicine, Cornell University, Ithaca, NY 14853, USA.
[b]Diagnostic Laboratory, College of Veterinary Medicine, Cornell University, Ithaca, NY 14853, USA.

RESULTS AND DISCUSSION

The proportion of non-*E risticii*-vaccinated horses testing positive for serum antibodies directed against *E risticii* was 7.3%. The county-specific seroprevalence ranged from 0-27%, with higher risk counties located at low elevation. At the farm level, an increasing width of nearby (within 2000 meters) creeks/rivers or lakes/swamps and increasing farm elevation were all associated with a reduction in the risk of exposure. An increasing distance between the horse and lakes/swamps was associated with an increase in the risk of exposure. Therefore, farms at higher risk were located predominately at low elevation with no running or standing bodies of water nearby. This finding was contrary to the opinion that exposure to this causative agent appeared to be associated with proximity to large river systems (Perry, 1986; Schmidtman et al., 1987). The flora surrounding the farm or within the pasture was not associated with exposure.

The risk of exposure to *E risticii* was 2-3 times higher for horses who had no access to a stall or run-in (therefore out 24 hours a day) compared to horses with access to a run-in shed that was cleaned out less than once a week. Application of fly spray only 2-3 times per week compared to daily application was associated with a 2-3 fold increase in the risk of exposure. While worming with benzimidazole-based wormers increased the risk of exposure, worming with pyrantel-based wormers decreased the risk of exposure. These associations between worming and exposure depended on the length of residency at the farm. Among horses born on the property, the risk of exposure was negatively associated with the number of horses turned out together.

Standardbred horses were 2-3 times more likely to have been exposed compared to thoroughbred horses, regardless of residency. Although male and middle-age horses were at a 1.5-3.0 times higher risk of exposure compared to other horses, age was significant only for those horses born on the property and sex was significant only for the first two cohorts of horses, ie. all horses or those horses born on the property or having lived on the property at least four years.

Horses tested during January through March were up to 6 times more likely to test seropositive compared to horses tested during July through September. Restricting the analysis to 4 year minimal residents or to horses born on the property enlarged the high risk season to include October-December. This higher risk for testing seropositive against *E risticii* during late fall through early spring is in agreement with our previous work done on horses sampled during 1985-86 (Atwill et al., 1992), but is in sharp contrast to other studies that have found the incidence of EME (Perry et al., 1986) and the risk of seropositivity (Goetz et al., 1989) to be highest during summer.

CONCLUSION

Farms at higher risk were located predominately at low elevation with no running or standing bodies of water nearby. The association of fly spray and access to a run-in shed with reduced risk of exposure supported the hypothesis that arthropods transmit this agent. Contradictory evidence for the arthropod hypothesis was the association of late fall to early winter with a higher risk of seropositivity, lack of an association between the surrounding farm-level flora and exposure, and why internal parasites were associated with exposure if arthropods are the sole vector for this disease. The association between internal parasiticides and exposure suggested that helminths may play a role in *E risticii* transmission. Contradictory evidence was the protective effect or lack of an effect between equine density and exposure, lack of an association between pasture or paddock characteristics and exposure, and why fly spray had any association at all if helminths are the only vector for this disease.

REFERENCES

Atwill, E.R., Mohammed, H.O., Dubovi, E.J. and Lopez, J., 1992. Retrospective evaluation of factors associated with the risk of seropositivity to *Ehrlichia risticii* in horses of New York state. Am. J. Vet. Res.,53: 1931-1934.

EGRET Statistical Package User's Manual, 1990. Statistics and Epidemiology Research Corporation (SERC). Software Division, Seattle, WA.

Goetz, T.E., Holland, C.J., Dawson, J.E., Ristic. M., Skibbe, K., Keegan. K.G., Johnson, P.J., Schaeffer, D.J. and Baker, G.J., 1989. Monthly prevalence (in 1986) of antibody titers against equine monocytic ehrlichiosis in apparently healthy horses in Illinois. Am J Vet Res, 50: 1936-1939.

Hahn, N.E., Fletcher, M., Rice, R.M., Kocan, K.M., Hansen, J.W., Hair, J.A., Barker, R.W., Perry, B.D., 1990. Attempted transmission of *Ehrlichia risticii* causative agent of Potomac Horse Fever by the ticks *Dermacentor variabilis, Rhipicephalus sanguineus, Ixodes scapularis, Amblyomma americanum*. Exp. Appl. Acarol., 8: 41-50.

Holland, C.J., Ristic, M., Cole, A.I., Johnson, P., Baker, G. and Goetz, T., 1985. Isolation, experimental transmission, and characterization of causative agent of Potomac horse fever. Science, 227: 522-524.

Knowles, R.C., Anderson, C.W., Shipley, W.D., Whitlock, R.H. and Perry, B.D., 1983. Acute equinè diarrhea syndrome (AEDS): a preliminary report. Proc. Am. Assoc. Equine Pract., 29: 353-357.

Palmer, J.E. and Benson, C.E., 1988. Oral transmission of *Ehrlichia risticii* results in Potomac horse fever. Vet. Rec., 122: 635.

Perry, B.D., Palmer, J.E., Troutt, H.F., Birch, J.B., Morris, D., Enrich, M. and Rikihisa, Y., 1986. A case-control study of Potomac horse fever. Prev. Vet. Med., 4: 69-82.

Pretzman, C., Rikihisa, Y., Ralph, D., and Fuerst, P., 1992. 16*S* rRNA sequence comparison between *Neorickettsia helminthoeca* and *Ehrlichia* spp. Proc. 92nd Gen. Meet. Am. Soc. Microbiol., 1992; 197.

Rikihisa, Y. and Perry, B.D., 1985. Causative ehrlichial organisms in Potomac horse fever. Infec. Immun. , 49: 513-517.

Rikihisa Y., 1991. Cross-reacting antigens between *Neorickettsia helminthoeca* and *Ehrlichia* species, shown by immunofluorescence and western immunoblotting. J. Clin. Micro., 29: 2024-2029.

Ristic, M., Holland, C.J., Dawson, J.E., Sessions, J. and Palmer, J.E., 1986. Diagnosis of equine monocytic ehrlichiosis (Potomac horse fever) by indirect immunofluorescence. J. Am. Vet. Med. Assoc., 189: 39-46.

Schmidtmann, E.T., Roble M.G., Carroll, J.F., 1986. Attempted transmission of *Ehrlichia risticii* by filed-captured *Dermacentor variabilis*. Am. J. Vet. Res., 7: 2393-2395.

Schmidtman, E.T., Rice, R.M., Roble, M.G. and Azad, A.F., 1987. Search for an arthropod vector of *Ehrlichia risticii*. Proc. Symp. Potomac horse fever, Schering Animal Health, Louisville, Kentucky, May, 1987. p 9-16.

Ziemer, E.L., Whitlock, R.H., Palmer, J.E. and Spencer, P.A., 1987. Clinical and hematologic variables in ponies with experimentally induced equine ehrlichial colitis (Potomac horse fever). Am. J. Vet. Res., 48: 63-67.