MONITORING PARASITE ACTIVITY AND DISEASE IN THE ROCKY MOUNTAIN BIGHORN BY ELECTROPHORESIS OF SEROMUCOIDS

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Source: Journal of Wildlife Diseases, 6(2) : 104-106

Published By: Wildlife Disease Association

URL: https://doi.org/10.7589/0090-3558-6.2.104

MONITORING PARASITE ACTIVITY AND DISEASE IN THE ROCKY MOUNTAIN BIGHORN BY ELECTROPHORESIS OF SEROMUCOIDS

The widespread respiratory diseases of Rocky Mountain bighorn sheep represent very complex etiologies with the possible involvement of helminth, viral and bacterial agents. Synergism between pathogens, although never demonstrated in this system, has been suggested in humans and domestic stock (Campbell and Martin, 1968, Vet. Rec. 82: 777-778; Woodruff, A. W., 1968, Trans. R. Soc. Trop. Med. Hyg. 62: 446-452).

Subclinical disease is difficult to monitor, particularly in a wild population. Extensive studies in man and experimental animals have shown that plasma glycoprotein increases significantly in a number of pathological states, including cancer and sterile inflammation (Shetlar, M. R. 1966, Progress in Clinical Pathology V. 1: 419-457; Winzler and Bekesi, 1967, Methods in Cancer Research V. 2: 159-202). These proteins, the "acute-phase reactants", respond relatively nonspecifically in injury, with the seromucoid fraction (protein soluble in 0.6 M perchloric acid) being the most affected.

In a study of the immune response of mountain sheep to their lungworm parasite, *Protostrongylus stilesi*, a method was required to give a direct measure of host-parasite interaction. This preliminary report indicates that certain acid soluble serum proteins can be used to monitor inflammation in the parasitized animal.

Methods

Seromucoids were extracted from serum and submitted to disc electrophoresis using a procedure modified from Price *et al* (1966, Am. J. Epidemiology 83: 152-175). The seromucoid fraction was prepared by diluting 2 ml of plasma with an equal volume of distilled water. After stirring and equilibration to 4°C, 2 ml of 1.8 M perchloric acid was added. Following neutralization of the supernate with KOH and dialysis against distilled water, the sample was subjected to electrophoresis on a 7% standard disc gel prepared from premixed stock solutions (Canalco). The stained gels were scanned by a microdensiometer (Joyce Loebel Chromoscan) and the relative protein concentrations were estimated by peak area.

Fecal larval counts were made at irregular monthly intervals from single fecal samples. Although Baermann's technique is customarily used for larval counts, a modified zinc sulfate flotation method (Faust *et al.*, 1939, J. Parasitol. 25: 241-262) was chosen to derive comparative values for several parasites. Only the lungworm larval counts were considered in this study.

Results and Discussion

Pathological changes in the profile of acid soluble proteins were expressed as the ratio of the electrophoretically fastest protein, tentatively identified as orosomucoid, to an unidentified β-globulin which appeared relatively unaltered by tissue injury (Figure 1). Using this index, definitive changes were noted during periods of increased parasite activity in two captive bighorns under observation (Figure 2).

A number of mechanisms have been proposed for cyclic parasite activity. The spring rise has been attributed to in-

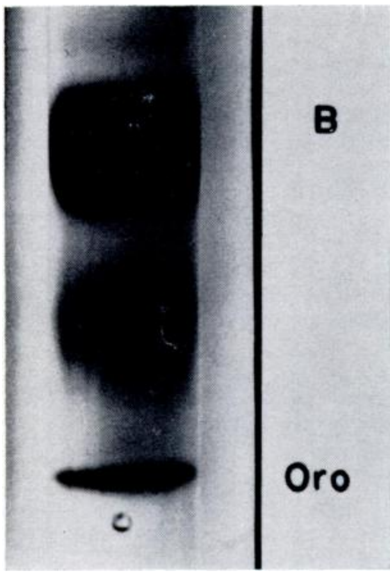

FIGURE 1. *Disc electrophoresis of acid soluble serum protein showing the two proteins, orosomucoid (Oro) and B, used to calculate the seromucoid index. The anode is located at the bottom of the figure.*

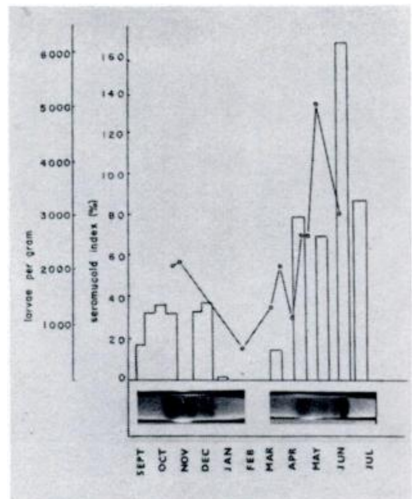

FIGURE 2. *The seromucoid response to parasite activity in a captive bighorn ewe. Parasite activity is represented by the frequency polygon and the seromucoid index by the bar graph. The stained disc gels demonstrate an increased proportion of orosomucoid, the band on the far right of each gel, in the spring rise.*

creased fecundity of adult worms already present, the maturation of dormant larvae, or the acquisition of new infection (Connan, R. M., 1969, World Rev. Animal Prod. 4: 53-58). Since the animals observed in this study were stabled, reinfection does not appear to provide a satisfactory explanation. The mechanism of host immunity to gastrointestinal nematodes appears to involve a reaginic (homocytotropic) antibody producing local anaphylaxis in the vicinity of the parasite lesion, permitting the passage of antibodies from the circulation (Barth *et al*, 1966, Immunology 10: 459-464). The seromucoid index may reflect a similar anaphylactic process in immunity to the lungworm. The homocytotropic antibody mediating this allergic inflammation is presently being studied.

Changes in seromucoid during acute bacterial infection were also noted. Twelve captive bighorns were studied during an outbreak of *Escherichia coli*. Early in the course of disease, the seromucoid index was elevated, but with the onset of scouring the orosomucoid band completely disappeared with a concomitant increase in a component of slower mobility (Figure 3). Large amounts of undegraded orosomucoid were recovered from diarrheic feces. When scouring was controlled by nitrofurazone, orosomucoid rapidly regained elevated concentrations. Loss of plasma protein is known to occur in severe diarrhea and other conditions associated with increased vascular permeability (Waldman, T. A., 1966. Gastroenterology 50: 422-443). This has been reported for IgG in the bovine

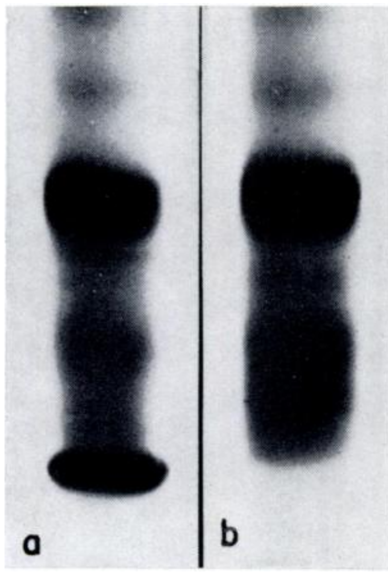

FIGURE 3. *Changes in the seromucoid profile with acute bacterial scours. a. phase of acute inflammation. b. phase of severe scouring showing the elimination of orosomucoid and the increase of a protein of next fastest mobility. The anode is located at the bottom of the figure.*

(Nielsen and Nanson, 1967. Can. J. Comp. Med. Vet. Sci. 31: 106-110), but the effect is often greater for proteins of low molecular weight such as albumin or orosomucoid. This selectivity has been demonstrated in certain nephrotic syndromes (Lines, D. R., 1969, Arch. Dis. Childh. 44: 461-464). The serum immunoglobulins determined by quantitative immunoelectrophoresis (Hudson *et al*, Amer. J. Vet. Res., in press) did not shcw a similar clear association with scouring intensity.

The orosomucoid index appears to respond very rapidly to tissue injury. Herzber *et al* (1967, Clin. Chem. 13: 1965-1070), noted a response to bone fractures in man within several days. Mia *et al* (1967 Am. J. Vet. Res. 28: 503-505) have reported rapid turnover rates of serum glycoproteins in domestic sheep. Nutritional stress appears to have only a minor effect on the response of acute-phase reactants to inflammation (Weimer and Godfrey, 1965, Can. J. Physiol. Pharmacol. 41: 925-935).

These preliminary observations indicate that seromucoids may be of value in monitoring subclinical disease in free-ranging animals.

Acknowledgements

The authors would like to acknowledge Miss Anne Thomas and Mr. David Kitts for performing the fecal larval counts and Miss Val Curylo for assisting with the electrophoresis.

This study was supported by the National Research Council of Canada.

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