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SERUM PROTEIN ELECTROPHORESIS IN BIGHORN SHEEP WITH CHRONIC PNEUMONIA

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Abstract: Sera from normal bighorn sheep *(Ovis canadensis)* and bighorns with chronic pneumonia attributed to *Mycoplasma* and bacteria were analyzed by cellulose acetate electrophoresis. Diseased sheep had significantly ($p = <0.001$) lower albumins, higher $\alpha 1$ and γ globulins, and A/G ratio below 1.0. The electrophoretic patterns, while not disease specific, provide means for detecting sub-clinical chronic disease and monitoring responses to therapy in bighorn sheep.

INTRODUCTION

Electrophoresis of serum proteins and analysis of the five major fractions provides a recognized means for detecting a variety of pathological processes in mammals.[10] Some disorders such as multiple myeloma produce diagnostic electrophoretic patterns[10] whereas others, including infectious diseases[10] and parasitic infestations,[1,6,11,12] are characterized by nonspecific patterns.

Lung diseases are a major problem affecting the health of bighorn sheep *(Ovis canadensis),* both free-ranging and in captivity.[2] The etiology of the diseases has not been clearly defined although investigators variously incriminated lungworms,[2] bacteria[8] and Mycoplasma.[13] Viruses have been suspect, but never demonstrated beyond question.[3]

The present study reports serum protein data from healthy and chronically diseased captive bighorns. The investigation was undertaken to determine the value of serum protein electrophoresis as a method for detection and monitoring chronic disease, predominantly lung disease, in bighorns.

MATERIALS AND METHODS

Blood collected serially from 13 bighorn sheep at the Rachelwood Wildlife Research Preserve, New Florence, Pennsylvania was allowed to clot for 4-6 hours and the serum was stored at —20 C. Total serum protein concentrations were determined with an American Optical Company TS Meter. Electrophoresis was performed on cellulose acetate using a Beckman Microzone apparatus and Ph 8.6 Beckman B-2 barbital buffer. After staining with Ponceau S, the strips were scanned in a Beckman Analytrol and the percentage of each fraction was calculated from the automated integrations of the area under the densitometer curve. Fractions were arbitrarily designated albumin and alpha-1, alpha-2, beta, and gamma globulin in order of fastest to slowest mobilities; two fractions migrating more slowly than the discreet beta globulin were included together as the gamma globulin component (Fig. 1). Albumin: Globulin (A/G) ratios were computed from the electrophoretic scan. Total leukocyte (WBC) counts were determined using a standard hemocytometer.

Sera were divided into two categories for statistical computation of mean values, standard deviations and p values (Table 1): 1) sera from sheep that remained healthy throughout the study period and sera obtained at times when sheep were clinically healthy comprised the normal group; 2) sheep with clinical evidence of chronic pneumonia constituted the diseased group. Two additional specimens that displayed either normal proteins dur-

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ing the time they had evidence of chronic pneumonia or displayed abnormal proteins associated with chronic disease of different etiology (nephritis of unknown cause, colitis, reticular impaction) were excluded from the statistical comparisons. The data were excluded since the intent of the comparisons were to determine mean values for normal proteins versus abnormal proteins associated with apparent and known pulmonary disease.

Evaluation of the clinical status of the sheep was made by physical examination. The presence of a nasal discharge, cough, and weight loss of varying severity were reliable signs of chronic pulmonary disease, a diagnosis further substantiated in many specimens at necropsy by gross, microscopic, and microbiological culture techniques (Woolf and Kradel, in prep.). More detailed hematology studies are also reported in that paper.

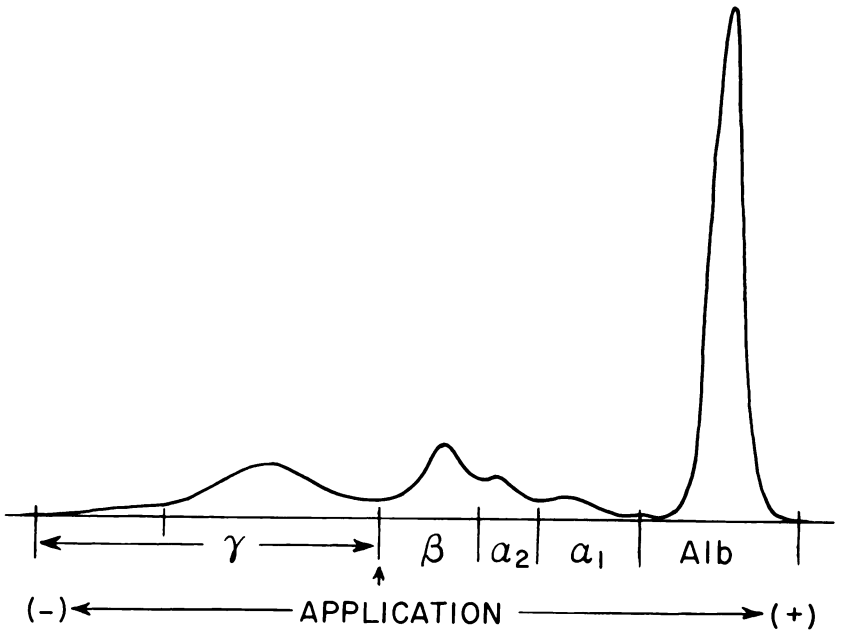

FIGURE 1. Electrophoretic pattern of a normal bighorn sheep.

TABLE 1. Comparison of mean serum protein values from normal sheep and abnormal values from those with chronic pneumonia.

	No. Sera (n)	Total Protein (gm%)	Albumin %	α_1 (%)	α_2 (%)	β (%)	γ (%)	A/G
Normal Sheep	24	7.06	55.41	3.57	5.61	12.54	22.85	1.20
Abnormal Sheep	20	7.77	39.36	4.90	6.79	14.55	34.43	0.66
		$p = <$ 0.025	$p = <$ 0.001	$p = <$ 0.001	$p = <$ 0.02	$p = <$ 0.02	$p = <$ 0.001	$p = <$ 0.001

RESULTS

The total serum proteins, A/G ratio, and percentages of albumin, and $\alpha 1$, $\alpha 2$, β, and γ globulins of all sheep were recorded along with pertinent clinical data and total WBC counts.

In Table 1 mean protein values and standard deviations of normal bighorns are compared with those of bighorns manifesting the chronic pneumonia syndrome. Chronic pneumonia in our bighorn sheep was associated with: a low serum albumin, below 50%; a moderate increase in α_1 globulin; a striking increase in the γ globulins, usually above 27%; and a reversal of the A/G to values below 1.0. These changes are highly significant ($p = <0.001$). The higher total proteins, α_2 and β globulins observed in chronically ill sheep were less significant.

The mean WBC of normal sheep was 9,312 (SD = 3,510) as compared to a mean WBC of 15,591 (SD = 4,394) in the sick group of bighorns. This difference was highly significant ($p=<0.001$).

Protein values of healthy sheep less than 1 year old were compared with adults and there were no statistically significant age differences ($p = <0.200$ - 0.975).

Variations in serum protein levels correlated closely with the clinical status of the sheep and were often a more sensitive indicator of disease than the total WBC count. For example, one sheep was clinically healthy with normal protein fractions but had an elevated WBC count whereas two other sheep both sick, had altered protein fractions, but normal WBC counts. In four sheep a return of serum protein fractions to or toward normal paralleled clinical signs of improving health such as decreasing nasal discharge and cough, and weight gain.

One sheep developed a low grade chronic illness associated with leukocytosis and eventually died of chronic pneumonia but did not exhibit protein abnormalities. This indicates the need for combining WBC counts with protein analyses when monitoring sheep for disease.

Another sheep was found to have chronic nephritis, colitis and reticular impaction, but had serum protein values similar to those of sheep with chronic pneumonia. Pathological evidence of the latter disease was not found.

DISCUSSION

Serum protein values of normal domestic and wild sheep vary widely depending on the methods utilized in each study and these variations must be considered before attempting to detect the presence of disease in sheep.[5,6,7] Our normal values, representing 24 serum samples from 12 bighorn sheep, agree with the higher percentages cited by Koenig et al.[5] and the results of Kuttler and Marble,[6] except for the α_2 and β globulins. They do not agree with the low albumin levels and high γ globulin values obtained with certain electrophoretic conditions used by Koenig et al.,[5] which are in our pathological range. Our values also agree closely with those of McGlinchy[7] who studied the sera of four healthy bighorn hybrids by electrophoresis on cellulose acetate, using different apparatus. As Sandor[10] pointed out, it is important for each laboratory to establish its own set of standards for each taxon under study, especially before attempting to make interspecific comparisons or analyzing animals for diseases.

Chronic pulmonary disease in bighorn sheep may be due to a variety of etiological agents acting alone or in combination. Forrester[2] reviewed the bighorn sheep lungworm-pneumonia complex due to infestation by *Protostrongylus* and called attention to the frequent association of this infestation with bacterial pathogens of the genera *Pasteurella* and *Corynebacterium*. Other workers recognized primary pulmonary infections due to *Pasteurella*.[8] The sheep used in this study had pulmonary infections from which *Mycoplasma* (PPLO) and a variety of bacteria were isolated.[13]

Sheep with lungworm infestations exhibited serum protein alteration characterized by decreased serum albumin and an increase γ globulin.[9] Mild infestations by *Protostrongylus* in bighorns may produce no serum protein alterations.[7] In lambs harboring *Haemonchus* and *Nematodirus* in their intestinal tracts, there was a statistically significant decrease in serum

albumin and an increase in the α_1, α_2, β and γ globulins as compared to non-parasitized lambs.[6] There are no published descriptions of serum proteins from sheep with chronic pneumonia due to bacteria or viruses.

Our bighorns with chronic pneumonia displaying low albumins, elevated α_1 and γ globulins, and A/G ratios less than 1.0 cannot be distinguished from sheep with parasitic infestations[6,9] and instead, these findings must be regarded as indications of chronic disease, a view substantiated by the occurrence of similar protein values in one of our sheep which died with chronic nephritis, colitis and reticular impaction.

We recognize that physiologic responses to factors such as level of nutrition or excitement can alter serum protein values. These sheep were maintained on the same diet and consumption did not vary throughout the study. Also, the bighorns were used to handling, and excitement or stress during blood sampling was minimal. While we cannot be certain that complex physiologic responses did not act independently of disease to produce the observed changes, the reported findings appear to be consistent in spite of other possible influences.

Chemical determinations of total protein, albumin, and globulin were also conducted on the same sera (Woolf and Kradel, in prep.). The values did not correspond to the electrophoretic determinations in all cases, but the same trends were apparent. We considered the electrophoretic determinations more accurate, but chemical determinations of A/G ratios would suffice as a diagnostic aid.

Despite the non-specificity of these protein patterns, serum protein electrophoresis provides, in conjunction with WBC counts, a simple means for evaluating the clinical status of wild sheep. This technique may serve to identify clinically inapparent disease in wild-trapped sheep prior to transplanting. It also has value in monitoring the condition of captive sheep in research stations and zoos. Both cellulose acetate electrophoresis and WBC counts are easier to perform on a routine basis than the seromucoid assay suggested by Hudson et al.[4] as a method for monitoring subclinical parasitic disease in bighorn sheep.

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LITERATURE CITED

1. DJAFAR, M. I., L. E. SWANSON, and R. B. BECKER. 1960. Clinical and hematological studies on pure *Dictyocaulus viviparus* (Block) lungworm infections in calves. J. Amer. vet. med. Assoc. 136: 200-204.
2. FORRESTER, D. J. 1971. In: *Parasitic Diseases of Wild Animals.* Iowa State University Press, Ames., J. W. Davis, and R. C. Anderson, editors.
3. HOWE, D. L. 1964. Etiology of pneumonia in bighorn sheep. Fed. Aid Div. Quart. Rept., Wyo. Dept. of Game and Fish. 16 p.
4. HUDSON, R., W. D. KITTS, and P. J. BANDY. 1970. Monitoring parasite activity and disease in the Rocky Mountain bighorn by electrophoresis of seromucoids. J. Wildl. Dis. 6: 104-106.
5. KOENIG, V. L., J. D. PERRINGS, and F. MUNDY. 1949. Electrophoretic analysis of lamb and sheep plasmas and sera. Arch. Biochem., New York. 22: 377-385.
6. KUTTLER, K. L., and D. W. MARBLE. 1960. Serum protein changes in lambs with naturally acquired nematode infections. Amer. J. vet. Res. 21: 445-448.

7. McGLINCHY, SARA E. 1971. Effects of *Protostrongylus stilesi* infection on bighorn X mouflon hybrids. M.S. Thesis, Colorado State University, Fort Collins.
8. POST, G. 1962. Pasteurellosis of Rocky Mountain bighorn sheep *(Ovis canadensis canadensis)*. Wildl. Dis. 62: 63.
9. POYNTER, D., and S. SELWAY. 1966. Diseases caused by lungworms. Helminth. Abst. 35: 105-127.
10. SANDOR, G. 1966. *Serum Proteins in Health and Disease*. Williams and Wilkins Co., Baltimore. 768 p.
11. SHARMA, K. M. L. 1967. Observations made on haematological and electrophoretic analysis of serum protein from heifers naturally infected with lungworm *(Dictyocaulus viviparus)*. Indian vet. J. 44: 489-493.
12. WILSON, G. I. 1961. Serum protein changes in lambs and kids after exposure to the thread lungworm, *Dictyocaulus filaria*. J. Parasit. 47: 20-21.
13. WOOLF, A., D. C. KRADEL, and G. R. BUBASH. 1970. *Mycoplasma* isolates from pneumonia in captive Rocky Mountain bighorn sheep. J. Wildl. Dis. 6: 169-170.

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