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FURTHER OBSERVATIONS ON *Protostrongylus* sp. INFECTION BY TRANSPLACENTAL TRANSMISSION IN BIGHORN SHEEP*

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Abstract: Tissues from two bighorn sheep fetuses and four neonatal bighorn sheep lambs were examined for evidence of natural *Protostongylus* infection occurring by the transplacental route. Forty-three third-stage larvae (L_3) were recovered from the fetal liver of case 1; one L_3 from the cotyledons of case 2; 189 L_3 from the liver and 46 L_3 from the lungs of case 3; 146 L_3 from the liver and 43 L_3 from the lungs of case 4; and 133 L_3 from the liver and 33 L_3 from the lungs of case 5. Fifty-two L_3 and fourth-stage larvae (L_4) were recovered from the lungs and five L_3 from the liver of case 6. Digestion with Pepsin-HCL was a more effective method for recovery of L_3 than baermannization, but L_4 larvae were digested.

INTRODUCTION

For a number of years a considerable amount of evidence has been compiled that *Protostrongylus* infection by the transplacental route occurred in bighorn sheep.[1,3,4,5,6] This evidence was all circumstantial until infective larvae were found in the liver and lungs of a near-term fetus from a ewe collected on Pike's Peak in Colorado.[2] Following this discovery, an effort was made to obtain additional proof for naturally occurring infection by the transplacental route.

MATERIALS AND METHODS

All spontaneous cases of dead or dying pregnant bighorn sheep, and neonatal lambs were given a complete postmortem examination to determine the cause of death. Unless otherwise indicated, the pathologist preserved 25% (by weight) of the liver and lungs from fetal (case 1, 2) or neonatal bighorn sheep (cases 3-6); the remaining 75% was made available for recovery of *Protostrongylus* larvae. A small amount of tissue from several lymph nodes was also examined, but the origin and amount of these lymph nodes was not consistent from case to case.

The liver and lungs available for recovery of larvae were finely minced into pieces varying from 1 to 5 mm in diameter and placed in a baermann funnel for 24 hours. Cases 1, 2 and 3 were examined only by this procedure; however, in all subsequent cases, the finely minced tissues after baermann funnel extraction were then digested with pepsin-hydrochloric acid.

RESULTS

Case #1:

On March 1, 1973, a 4-year-old ewe accidentally died during routine trapping operations on Pike's Peak in Colorado. Measurement of the fetus indicated she was 4.5 to 5 months pregnant. The cotyledons, fetal liver, fetal lungs and a small amount of fetal lymph nodes were examined for L_3 *Protostrongylus*. Forty-three L_3 of a *Protostrongylus* sp. were recovered from the fetal liver; none was found in the lungs, nymph nodes or cotyledons.

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Case #2:

On April 6, 1973, a 4-year-old captive ewe obtained from Pike's Peak died with clinical signs of pasteurellosis and *Pasteurella hemolytica* was cultured from the lungs. Measurement of the fetus indicated she was 3.5 to 4 months pregnant. The cotyledons, fetal liver, fetal lungs and a small amount of fetal lymph nodes were examined for L_3 *Protostrongylus*. One L_3 was found in the cotyledons. None was found in any of the other tissues.

Case #3:

On April 29, 1973, a 5 year-old captive ewe obtained from Pike's Peak aborted a full-term ewe lamb. The ewe had been ill for several months with chronic pneumonia and was subjected to considerable physical abuse by the healthier animals in the herd. Forty percent of the liver and the right lung were examined for L_3 *Protostrongylus;* the remainder of the liver and the left lung were preserved for histopathologic examination. A total of 189 L_3 was recovered from the liver. The various lobes of the right lung were separated for examination and seven L_3 were found in the diaphragmatic, 17 in the apical and 22 in the cardiac and intermediate lobes (combined), making a total of 46 larvae.

Case #4:

On May 21, 1973, an 8 year-old captive ewe from Pike's Peak gave birth to a lamb that died 24 hours later with an enteric clostridial infection. Thirty-six L_3 were recovered from the lungs and 22 from the liver by the baermann funnel technique. An additional 110 L_3 were recovered from the lungs and 21 from the liver by digestion.

Case #5:

On May 27, 1973, an 8 year-old captive ewe from Pike's Peak gave birth to a lamb that died of starvation 2.5 to 3 days later. Thirty L_3 were recovered from the lungs and 19 from the liver by the baermann funnel technique. An additional 103 L_3 were recovered from the lungs and 14 from the liver by digestion.

Case #6:

On May 14, 1973, a ewe lamb estimated to be 24 to 36 hours old was captured during routine work on Pike's Peak. Seven days later she died with an enteric clostridial infection. Thirty-nine larvae, with essentially equal numbers of late 3rd stages and early 4th stages, were recovered from the lungs, but none was found in the liver using the baermann funnel technique. An additional 13 late L_3 were found in the lungs and five infective L_3 were found in the liver by the digestion technique. A large number of partially to almost totally digested fourth stage (L_4) larvae were evident in the digesta from the lungs. It was not possible to estimate the number of these larvae that were recovered.

DISCUSSION

The results obtained from fetal and neonatal bighorn sheep indicate that the transplacental route is the usual means by which lambs become infected with *Protostrongylus,* at least in the Pike's Peak herd. The species of *Protostrongylus* that is transplacentally transmitted is probably *Protostrongylus stilesi.* Postmortem examination of 33 lambs, 2 weeks to 4 months old, did not reveal *Protostrongylus rushi;* however, this parasite does occur in yearlings and adults on Pike's Peak. Identification of the *Protostrongylus* larva will be determined by experimental infection.

It could be suggested that transplacental transmission begins in the 4th month of pregnancy and continues until birth, but the six cases reported here are insufficient to make any final conclusion. Moreover, measurement of the fetus to estimate fetal age is not very accurate for bighorn sheep.

The data obtained from cases 4 and 5 indicate that the baermann funnel technique is a poor procedure for the recovery of L_3 *Protostrongylus.* These lar-

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vae are extremely lethargic and are probably trapped in the tissues; moreover, finely minced tissues tend to clog the screens, effectively inhibiting passage of larvae that have escaped the tissues. Thus we must conclude that the number of larvae obtained from the case reported last year, and the first three examined this year, represent only a fraction of the total.

The pepsin-hydrochloric acid digestion also has drawbacks. While L_3 are impervious to digestion, L_4 and adults are highly susceptible. Lambs older than 4 days would probably be infected with some early L_4 and therefore results would be inaccurate.

Since a considerable amount of liver and lungs from these fetal and neonatal lambs was preserved for histopathologic examination, the actual infections were probably far greater than the data indicate. For example, in case 3, 189 L_3 were found in 40% of the liver and 46 L_3 in the right lung. Theoretically, this lamb had 472 in the liver and 92 in the lungs using only the baermann technique. If the tissues from this particular case had been digested following baermannization, the number theoretically possible would be even greater because digestion is at least 50% more effective for recovery of the L_3 stage.

Currently, an intensive effort is underway in Colorado to determine the cause of a lamb mortality that is affecting several herds. The problem is extremely severe in the Pike's Peak herd, with mortality reaching 95 to 98% within 3 months after the lambs are born. Pneumonia is responsible for this mortality, and present evidence indicates that transplacental infection with large numbers of lungworms is a predisposing factor. Experimental infection studies should make clear the role of *Protostrongylus* infection in mortality in young bighorn lambs.

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