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Source: Bulletin of the Wildlife Disease Association, 3(2) : 74-76

Published By: Wildlife Disease Association

URL: https://doi.org/10.7589/0090-3558-3.2.74

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Death of the snake was interpreted as the direct result of absorption through the mucous membranes of the mouth, esophagus, and stomach, of toxic secretions from skin glands of the toad. Absorption of a fatal dose of poison within two minutes attests to the potency of the toxin. It is of interest that the common toad is a preferred item of diet for the hognosed snake (*Heterodon platyrhynos*).

Schrietmuller and Lederer report poisoning of snakes by ingestion of the common European toad (*Bufo bufo*). (Schreitmuller, W. and Lederer, G., 1930. Krankheitserscheinungen bei Fischen, Reptilien and Lurchen. Wenzel. Berlin. : Cited by Reichenbach-Klinke, H. and Elkan, E. 1965. The principal diseases of lower vertebrates. Academic Press. New York. p. 527).

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23 December, 1966

AN ATTEMPT TO ISOLATE VIRUSES FROM LUNG TISSUE AND LUNG NEMATODES OF BIGHORN SHEEP

The reduction of populations of Rocky Mountain bighorn sheep (*Ovis c. canadensis*) has been documented and discussed by many authors (Hunter and Pillmore, 1954, Trans. No. Am. Wildl. Conf. 19: 117-129; Buechner, 1960, Wildlife Monographs 4: 94-110; Forrester and Senger, 1963, Mont. Wildlife, April: 2-7). Various disease conditions have been implicated as causes of this decline, but respiratory infections appear to be particularly important (Pillmore, 1958, Trans. Desert Bighorn Sheep Council: 57-63).

In recent years a lungworm-pneumonia complex has been suggested as a possible regulatory factor in populations of bighorn sheep (Buechner, *op. cit.*). The lung nematodes *Protostrongylus stilesi* and *P. rushi* are commonly associated with this complex (Buechner, *op. cit.*; Forrester and Senger, 1964, J. Wildl. Mgt. 28: 481-491), but the epizootic nature of the die-offs suggests that bacteria or viruses may be involved as well (Hunter and Pillmore, *op. cit.;* Pillmore, *op. cit.*). Marsh (1938, J. Mammal. 19: 214-219) and Post (1962, Wildl. Dis. 23: 1-14) have reported on bacteriologic studies of infections caused by *Pasteurella* and *Corynebacterium* spp. in bighorn sheep, and recently Howe *et al.* (1966, Bull. Wildl. Dis. Assoc. 2: 34-37) have published the first report on virologic studies. In the latter study significant antibody titers to bovine myxovirus parainfluenza-3 (PI-3) were found in sera from bighorn sheep in Wyoming and Montana, but no viral agents were isolated from nasal swabs and lung tissue cultured on bovine embryonic kidney (BEK) cell cultures.

The present study was undertaken in an attempt to isolate PI-3 viruses and/or other respiratory viruses from lung tissue and adult lungworms of apparently normal bighorn sheep in western Montana where pneumonia-like diseases are known to occur (Marsh, *op. cit.*).

The lungs of 22 adult male bighorn sheep which were killed by hunters during October and November of 1965 were collected by personnel of the Montana Fish and Game Department. Each lung was packaged individually and kept frozen until examined. The majority of the lungs (19) were from the Sun River herd; one lung was collected from the Kootenai Falls herd and two were taken from the Ural-Tweed herd (Fig. 1). Table 1 shows the number of lungs and the types of samples that were tested for virus. The lungs were shipped in frozen condition to California and kept in a mechanical freezer at -20°C until thawed and examined during January, February and March, 1966.

Lung tissue was cultured on BEK cell

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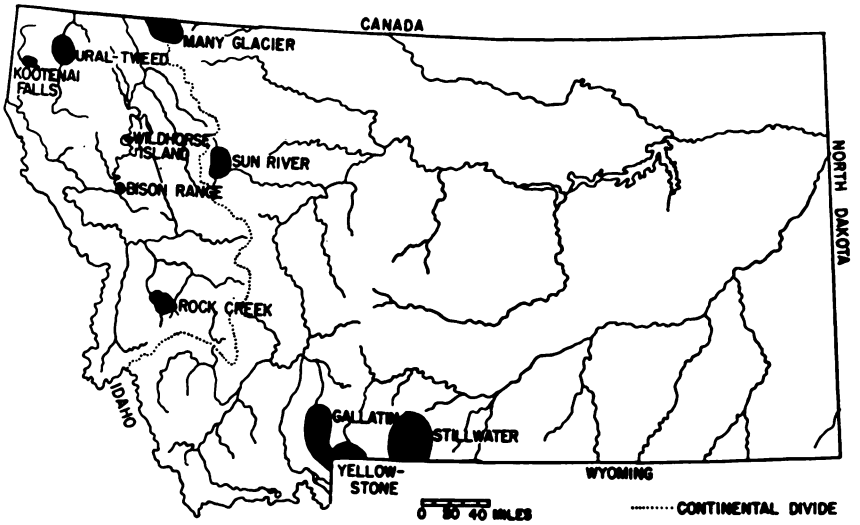

Figure 1. *Location of the ten largest bighorn sheep herds in Montana, three of which (Sun River, Ural-Tweed and Kootenai Falls) were sampled in the present study.*

cultures essentially as described by McKercher *et al.* (1963, Am. J. Vet. Res. 24: 510-514) for isolation of infectious bovine rhinotracheitis (IBR) virus. Fifty percent suspensions were prepared by mincing the lung tissue with scissors and grinding the minced material in Ten-Broeck grinders with equal parts (w/v) of Earle's lactalbumin medium. Suspensions were prepared only from consolidated lesion tissue (caused by *P. stilesi* and including adult nematodes, first stage larvae and ova) in the first ten lungs. For the remaining 12 lungs, normal (i.e. without gross, visible lesions) and consolidated tissue samples were cultured separately (Table 1). The inoculum, in dilutions of 10^{-1} and 10^{-2}, was added to tubes of BEK cell culture, using three tubes for each dilution. Tissue from three lungs was cultured also on lamb embryo kidney (LEK) cell cultures. The cultures were incubated in a stationary position at 37°C and were examined daily over a seven day period for cytopathic effects (CPE). After seven days' incubation the cell culture fluid was harvested, pools were prepared and two additional subpassages were made as described above. Two-tenths ml of 0.5 percent guinea pig red blood cells was added to the cell sheets and the cultures were examined microscopically for evidence of hemadsorption after

Table 1. *Numbers of lungs and types of material tested for respiratory viruses.*

Herd	Total number of lungs sampled	Number of lungs sampled via: *P. stilesi* lesions only	*P. stilesi* lesions and normal tissue	*P. rushi* adults
Ural-Tweed	2	2	0	0
Kootenai Falls	1	1	0	0
Sun River	19	7	12	14*
Totals	22	10	12	14*

* The total number of adult *P. rushi* sampled was 222. Numbers sampled per lung averaged 16 and ranged from 2 to 76 in 14 lungs from the Sun River herd.

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15 to 30 minutes incubation at room temperature. Adult lung nematodes (*P. rushi*) which had been recovered from the air passages of the lungs were cultured in the same manner as described above.

No cytopathic agents were isolated on the cell cultures. The inoculated cell cultures also failed to demonstrate the hemadsorption phenomenon. These results are in agreement with those reported by Howe (*op. cit.*) who also failed to isolate cytopathic agents from samples of lung tissue from 15 different bighorn sheep that had died in various stages of pneumonia. The validity of these two sets of negative results is open to some question. In the case of the Wyoming work, the lung tissue was stored at -15°C for as long as 18 months before testing for virus (Howe, 1965, Quart. Rep. Wyo. Game & Fish Comm., 21-24; Howe *et al.*, *op. cit.*). The lungs that were used in the present study were stored at -20°C for up to six months in some cases before the tissue was thawed and cultured. Lief (1966, Myxoviruses, Ch. 10, pp. 246-312, *Basic Medical Virology*, J. E. Prier, editor, Williams and Wilkins Co., Baltimore) reports that some myxoviruses survive freezing at -70°C or lyophilization for a long period, but that at 4°C and -20°C they may lose infectivity in a few days. It is possible, therefore, that any viruses that were present at the time of collection were no longer infective at the time of testing.

Additional attempts should be made to isolate respiratory viruses from bighorn sheep nasal swabs and lung tissue using fresh material in order to obtain more reliable results. Studies involving both bacteria and viruses should be continued and expanded to investigate the possibility of a virus-bacteria combined effect similar to that indicated for shipping fever in cattle (Hetrick *et al.*, 1963, Am. J. Vet. Res. 24: 939-947; Saunders *et al.*, 1964, Can. J. Comp. Med. Vet. Sci. 28: 57-62).

This study was supported in part by Graduate Training Grant 5T1-762 from the U.S. Public Health Service. We wish to thank the personnel of Montana Fish and Game Department, particularly Mr. Wynn G. Freeman, for help in obtaining the lungs and Dr. D. G. McKercher, Department of Veterinary Microbiology, University of California, Davis, for providing advice and facilities for the cell culture work.

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NOTES ON THE HOST SPECIFICITY OF *Corynosoma hamanni* (LINSTOW, 1892)[1].

Spiny-headed worms of the genus *Corynosoma*, Polymorphidae, present an interesting problem in host specificity as they are the only acanthocephalans which have both aquatic birds and mammals as the normal definitive hosts. Our work on the life cycle of *C. hamanni*, coupled with a survey of Antarctic helminth parasites, has produced data on the host specificity of this species.

Materials and Methods

Amphipods of the *Orchomenella plebs-rossi* complex were maintained in aquaria containing aerated sea water under controlled temperature conditions (-2 to 8°). Amphipods were exposed to *C. hamanni* eggs by three methods: maintaining the amphipods in sea water containing seal feces as a food source; exposing the amphipods to chopped female worms for one hour; exposing the amphipods for one hour to eggs removed from the body cavity of female worms. Amphipod intestines were removed according to the technique described by De Giusti (1949. J. Parasit. 35: 437-460) and examined with the aid of a dissecting microscope at 80X magnification and/or a compound microscope at 100X magnification. Seven amphipods from the February batches and 25 amphipods from the September batches were sectioned and stained after preliminary examination.

[1] Supported in part by N. S. F. Grants #146 and #228.

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