

Expanded abstract

Protostrongylidae in Cervidae and *Ovibos moscatus*; a clustering based on isoelectric focusing on nematode proteins

M. Stéen¹, S. Persson² and L. Hajdu³

¹ The Swedish University of Agricultural Sciences, Department of Veterinary Microbiology, section of Parasitology, S-75007, Uppsala, Sweden.

² The Swedish University of Agricultural Sciences, Faculty of Forestry, S-75007, Uppsala, Sweden.

³ The National Veterinary Institute, S-75007, Uppsala, Sweden.

Key words: Bovidae, Cervidae, Numerical taxonomy, Protostrongylidae

Rangifer, 13 (4): 221–223

Introduction

The family Protostrongylidae Leipner, 1926 consists of many genera and species such as *Muellerius* spp. (Mueller, 1889). Cameron, 1927, *Capreocaulus* spp. Shultz and Kadenatsii, 1948, *Varestrongylus* spp. Bhalerao (1932) and *Elaphostrongylus* spp. (Cameron, 1931; Lybimov, 1945; Mitskevich, 1960; Stéen, Chabaud and Rehbinde, 1989). First stage larvae of the Protostrongylidae are difficult to distinguish morphologically. In adult protostrongylids, the male bursae and spiculae in particular have been used for taxonomical classification. The classification of adult and juvenile Elaphostrongylinae is also difficult. We attempted to identify juvenile Protostrongylidae and adult *Elaphostrongylus* using isoelectric focusing.

Materials and methods

First stage protostrongylid larvae were obtained from muskoxen (*Ovibos moscatus*), roe deer (*Capreolus capreolus*), and from lung granulomas

with taxonomical identified *Varestrongylus alces* from moose (*Alces alces*). First stage protostrongylid larvae were collected from moose positive for adult *Elaphostrongylus*, or from moose not known to be infected with adult *Elaphostrongylus*. Adult *Elaphostrongylus* from reindeer (*Rangifer tarandus*) and moose were also obtained. The specimens were not identified to species. Standard techniques were used to reveal the protein band pattern at their protein isoelectric points (pI) (Tegelström *et al.*, 1982; Tegelström *et al.*, 1983). An application program, ISOCALC, was used to determine the pI's (Johansson, 1988). Numerical analysis of the protein banding patterns was performed by clustering, and resemblance was calculated with Czekanowski's similarity measure (Czekanowski, 1909; Hajdu, 1981; Hajdu and Rajczyk, 1981; Hajdu, 1983). The similarity matrix was transformed by unweighted Pair Group Method with Arithmetic (UPGMA) fusion (Sneath and Sokal, 1973) to a dendrogram.

Results

The analysis revealed a close relationship between first stage protostrongylid larvae from muskoxen, roe deer and *Varestrongylus alces* first stage larvae. The first stage larvae of roe deer and *V. alces* were the most closely related. First stage protostrongylid larvae from moose and adult *Elaphostrongylus* in moose were related. Adult *Elaphostrongylus* from reindeer and moose showed similarity and at the same time distinct differences in their protein pattern.

Discussion

The family Protostrongylidae is defined morphologically by a dorsal spine at the tail in first stage larvae (Anderson, 1978). This dorsal spine occurs in the Nordic genera *Muellerius*, *Capreocaulus*, *Varestrongylus* and *Elaphostrongylus* which makes separating them difficult. Yamaguti (1961) and Anderson (1978) considered that *Capreocaulus* spp. and *V. sagittatus* were identical, while Kutzer and Prosl (1975) considered first stage larvae of *C. capreoli* to be distinguishable from both *E. cervi* and *V. sagittatus*. Demiaszkiewicz (1986) called the protostrongylid in roe deer *V. capreoli*, instead of *C. capreoli*. Lankester *et al.* (1976) stated that several first stage larvae of Protostrongylidae «can't be identified reliably even to genus», referring to Elaphostrongylinae, Muellerinae, Capreocaulinae and Neostongylinae. In the genus *Elaphostrongylus* Cameron, 1931 opinions vary as to the independent status of the species. Pryadko and Boev (1971) considered *E. cervi*, *E. rangiferi* and *E. panticola* to be subspecies of *E. cervi*. Recently, Stéen, Chabaud and Reh binder (1989) asserted that *Elaphostrongylus* in moose can be distinguished from other forms of *Elaphostrongylus* and should therefore be named *Elaphostrongylus alces*. Stéen and Johansson (1990) added complementary morphological descriptions of *E. alces*, *E. cervi* and *E. rangiferi*. Further studies have confirmed these results (Gibbons *et al.*, 1991; Stéen, 1991; Carreno and Lankester, 1993).

In our study, the protein profiles, the matrices and the dendrogram revealed differences and similarities among protostrongylids and among elaphostrongylines. The relationship between adult *Elaphostrongylus* and protostrongylid larvae from moose was seen. The specificity of adult *Elaphostrongylus* from moose and reindeer was also shown. Other methods such as isoen-

zymatic electrophoresis and/or DNA-techniques should be applied to confirm the results.

Acknowledgement

The study was financed by the Swedish Environmental Protection Agency.

References

- Anderson, R. C. 1978. Keys to genera of the superfamily Metastrongylidae. Farnham Royal Buks, U.K. no. 5: Commonwealth Agricultural Bureau. (eds. Anderson, R.C.; Chabaud, A.G. and Willmott, S.) - *CIH Keys to the nematode parasites of vertebrates*.
- Bhalerao, G. D. 1932. On some nematode parasites of goats and sheep at Muktesar. - *Indian Journal of Veterinarian Sciences Animal Husbandry* 2: 242-254.
- Cameron, T. W. M. 1927. Studies on three new genera and some little known species of the nematode family Protostrongylidae Leipner 1926. - *Journal of Helminthology* 5 (1): 1-24.
- Cameron, T. W. M. 1931. On two new species of nematodes from the Scottish red deer. - *Journal of Helminthology* 9 (4): 213-216.
- Carreno, R. A. and Lankester, M. W. 1993. Additional information on the morphology of the Elaphostrongylinae (Nematoda: Protostrongylidae) of North American Cervidae. - *Canadian Journal of Zoology* 71: 592-600.
- Czekanowski, I. 1909. Zur Differential diagnosis der Neandertalgruppe. - *Korrespondenzblatt Deutsche Anthropologen Gesichte* 40: 44-47.
- Demiaszkiewicz, A. W. 1986. Laboratoryjna diagnostyka roziacowa Protostrongylidow jeleniowatych (Laboratory diagnosis of Protostrongylidae in Cervidae). *Medycyna Weterynaryjna* 11-12: 660-663.
- Gibbons, L. M., Halvorsen, O. and Stuve, G. 1991. Revision of the genus *Elaphostrongylus* Cameron (Nematoda, Metastrongyloidea) with particular reference to species of the genus occurring in Norwegian cervids. - *Zoological Scripta* 20 (1): 15-26.
- Hajdu, L. and Rajczy, M. 1981. RISE analysis: a new method for interpretation of dendrograms. - *Proceedings of the 3rd Hungarian Biometric conference Budapest* 129-133.
- Hajdu, L. 1981. Graphical comparison of resemblance measures in phytosociology. - *Vegetatio* 48: 47-59.
- Hajdu, L. 1983. A chromosome inversion pathway for some *Chironomus* species and two new techniques for analyzing similarity functions. - *Numerical Taxonomy* vol. G1. (Ed. Felsenstein, I.) pp. 532-535, NATO ASI.

- Johansson, K.-E. 1988. ISOCALC Ver. 1.10, Isoelectric point estimation of proteins by isoelectric focusing with Pharmacia isoelectric kit (ph 3-10). - *National Veterinary Institute Box 7073, 75007, Uppsala, Sweden*.
- Kutzer, E. and Prosl, H. 1975. Zur Kenntnis von *Elaphostrongylus Cervi* Cameron 1931, 1: Morphologie und Diagnose. - *Wiener Tierärztliche Monatschrift* 62: 258-266.
- Lankester, M. W., Crichton, V. I. and Timmermann, H. R. 1976. A protostrongylid nematode of woodland caribou and implications in moose management. - *Proceeding of the 12th North American Moose Conference and Workshop St. John's Newfoundland, Canada*, 173-189.
- Leipner, R. T. 1926. On the roundworm genera *Protostrongylus* and *Angiostrongylus* of Kamensky 1905. - *Journal of Helminthology* 4 (4-5): 230-270.
- Lybimov, M. P. 1945. Novye glistnye zabolovaniya mozga pantovykh olenei (New helminth disease of the brain of izubra). - *Sbornik nauch nykh Issledovatel'skikh laboratoria Rabot. (Pantovogo olenovodstva NKSKH SSSR)*. Moscow, Izdatel'stvo Nkskh SSSR. 1: 225-232.
- Mitskevich, V. Yu. 1960. *Elaphostrongylus rangiferi* - novyi vid gel'minta olenya (*Elaphostrongylus rangiferi*, a new species of helminth of reindeer). - *Parazity Zhivotnykh i Prirodnaya Ochagovost'Boleznei Trudy In-ta Zool. AN Kaz SSR* 12: 115-119.
- Mueller, A. 1889. Die Nematoden der Säugetierlungen und die Lungenwurmkrankheit. - *Deutsche Zeitschrift für Tiermedizin* 12: 261-321.
- Pryadko, E. I. and Boev, S. N. 1971. Sistematika, filogeniya i evolyutsiya elafostrogilin - nematod olenei (Systematics, phylogeny and evolution of elaphostrongyline - nematodes of deer). - *Izdatel'stvo Akad. Nauk Kazakhskoi, SSR, Ser. B*, 5: 41-48.
- Schultz, R. S and Kadenatzii, A. N. 1948. (Morphology of the copulatory organs in the new nematode genus *Capreocaulus* from roe deer). - *Doklady Akademii Nauk SSSR* 63: 341-344.
- Sneath, P. H. A. and Sokal, R. R. 1973. Principals and practice of numerical classification. - *Numerical taxonomy*. 2nd edn., San Francisco. pp. 488-547.
- Stéen, M., Chabaud, A. G. and Rehbinder, C. 1989. Species of the genus *Elaphostrongylus* parasite of Swedish Cervidae. A description of *E. alces* n. sp. - *Annals de Parasitologie Humaine et Comperee* 64(2): 134-142.
- Stéen, M. and Johansson, C. 1990. *Elaphostrongylus* spp. from Scandinavian Cervidae - a scanning electron microscope study (SEM). - *Rangifer*, 10(1): 39-46.
- Stéen, M. 1991. Elaphostrongylosis - a clinical, pathological and taxonomical study with special emphasis on the infection in moose. - *Ph.D. thesis. Department of Veterinary Microbiology, Swedish University of Agricultural Sciences, Uppsala, Sweden*.
- Tegelström, H., Nilsson, G. and Wyöni, P.-I. 1983. Lack of species differences in isoelectric focused proteins in the *Formica rufa* group. (Hymenoptera, Formicidae) - *Hereditas* 98: 161-165.
- Tegelström, H., Wyöni, P. I. and Rytman, H. 1982. Thin layer isoelectric focusing as a tool for higher category systematics. - *Annales Zoologici Fennici* 19: 203-207.
- Yamaguti, S. 1961. Nematodes of vertebrates. - *Systema Helmintum Part I to V Vol. III: Interscience Publisher Inc., New York*, pp. 331-504.

Manuscript accepted 1 November 1993