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CHARACTERIZATION OF A *TRICHINELLA* ISOLATE FROM POLAR BEAR

by

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Summary — An isolate of *Trichinella* of polar bear origin was studied by isoenzymatic typing. It was found referable to *Trichinella nativa*. While the Wistar rats proved nearly refractory to this isolate, the Swiss albino mice were highly susceptible. Ninety-one per cent of the cystic lesions in the diaphragm of the polar bear contained viable larvae after over 20 years of acquisition of the infection by the host which is a case of extreme adaptability of the parasite to its host. The anatomo-pathological aspects of these lesions are studied and the zoonotic significance of this isolate examined.

KEYWORDS: *Trichinella*; Polar Bear; Isoenzyme Study; Laboratory Host Susceptibility; Zoonotic Potentiality

Introduction

A male polar bear, *Ursus maritimus* Phipps, born wild and captured at an unknown location in the artic at about seven months of age, was held captive as an exhibit at the Zoological Garden, Antwerp for over twenty years until its euthanasia in December, 1988. The animal had an incurable massive tumoral growth in the liver and it was considered inappropriate resorting to surgical measures. The growth was later attributable to hepato-cellular carcinoma. The animal also showed gross lesions of *Trichinella* sp. infection in the diaphragm. Since an arctic or subartic *Trichinella* sp. had been associated with a French epidemic of human trichinellosis (1, 9), this prompted the authors to study the biological and biocheminal characteristics of the nematode isolate and the anatomo-pathological aspects of the lesions.

Materials and methods

Infection of rats and mice

Each gram of minced trichinosed diaphragm of the polar bear was digested in 40 ml of artificial digestion fluid consisting of 1 % pepsin (EC 3.4.23.1, 1 :10,000) and the pH set at 3.0 with concentrated HCl. The digestion was carried out for four hours and the digest put to Baermann extraction for isolation of the free larvae. The recovered larvae were counted

and used for infecting young Wistar rats and Swiss albino mice. Two rats and five mice were each infected with 1000 and 500 larvae respectively by gavage. Thirty to 40 days later, these animals were sacrificed by cervical dislocation, skinned and eviscerated. Their carcasses were ground and digested as above and the recovered larvae were counted to compare the suceptibility of rats and mice.

Biochemical identification

The isoenzyme typing of the present *Trichinella* isolate (code MURS/BL/88/ISS135) was carried out according to the protocol of Pozio (5) and Pozio *et al.* (7). Briefly, the larvae at the second passage in Swiss albino mice were collected after artificial digestion, washed, homogenized in a ground glass minihomogenizer on ice and centrifuged for one hr at 5,500 r.p.m. at 4° C. The supernatant was utilized for electrophoresis. Three marker strains had been used: MSUS/PO/60/ISS3 for *T. spiralis* sensu stricto, MURS/NO/84/ISS10 for *T. nativa*, and MVUL/IT/82/ISS2 for *Trichinella* T3 (6).

The following 27 gene enzyme systems were examined: PGD (EC 1.1.1.8), LDH (EC 1.1.1.27), MDH (EC 1.1.1.37), ME (EC 1.1.1.40), IDH (EC 1.1.1.42), 6PGD (EC 1.1.1.44), G6PD (EC 1.1.1.49), G3PD (EC 1.2.1.12), GLDH (EC 1.4.1.3), SOD (EC 1.15.1.1), GOT (EC 2.6.1.1), ALAT (EC 2.6.1.2), AK (EC 2.7.4.3), PGM (EC 2.7.5.1), ES (EC 3.1.1.1), ACPH (EC 3.1.3.2), PEP1 (EC 3.4.11.1), PEP2 (EC 3.4.11.2), ADA (EC 3.5.4.4), ALDO (EC 4.1.2.13), CA (EC 4.2.1.1), FUM (EC 4.2.1.2), ACON (EC 4.2.1.3), ENOL (EC 4.2.1.11), TPI (EC 5.3.1.1), MPI (EC 5.3.1.8), GPI (EC 5.3.1.9).

The electrophoresis was carried out in 12% starch gel for 12 hr at 4° C. The details of electrophoretic and development conditions are mentioned in previous papers (5,7).

Anatomopathological studies

The pieces of diaphragm muscle were fixed in buffered 10% formalin, embedded in paraffin and the sections stained by conventional haematoxylin eosin or periodic acid-Schiff (PAS) methods.

Results

The digestion of the trichinosed diaphragm of the polar bear yielded 51 larvae per gram of muscle which is indicative of a moderate level of infection. The carcasses of the two rats, each infected with 1000 larvae of the original isolate, harboured an average of three larvae and showed a reproductive capacity index (RCI) of 0.003. In the Swiss albino mice infected with 500 such larvae each, a sustained level of muscle infection was obtained and the RCI reached 3.2. As the mouse host was very receptive to this isolate, the infection was subsequently maintained and propagated in Swiss mice by serial passage every one to two months.

The electrophoretic profile of the isoenzymes of the present isolate showed biochemical behaviour very similar to the reference strain of *Trichinel-*

la nativa, arctic species (code MURS/NO/84/ISS10) for 27 enzyme systems and was also identical to the isolate associated with the Parisian outbreak of trichinellosis.

The lesions in the diaphragm of the polar bear were grossly visible on the surface. These appeared as spindle shaped white specks, measuring 2-3 mm long and were discretely distributed over the entire surface. A majority of these lesions showed a single larva each when examined under low magnification of a microscope by muscle compression using a trichinoscope; only nine per cent of these cystic lesions contained amorphous material and did not show larvae. Histological sections of these lesions showed thick and, fibrous cyst-wall (Fig.1). There was no evidence of cellular infiltration of any type, neither in the cyst contents or its wall nor in the adjacent muscle tissue. The contents of the cyst showed larva cut at various planes. The larva, which showed strong PAS reaction, was lodged in a homogeneous acellular matrix. Only occasionally did the cyst content show scar tissue and calcification was very rare. The muscle tissue in the vicinity of the cystic lesions did not show any alteration.

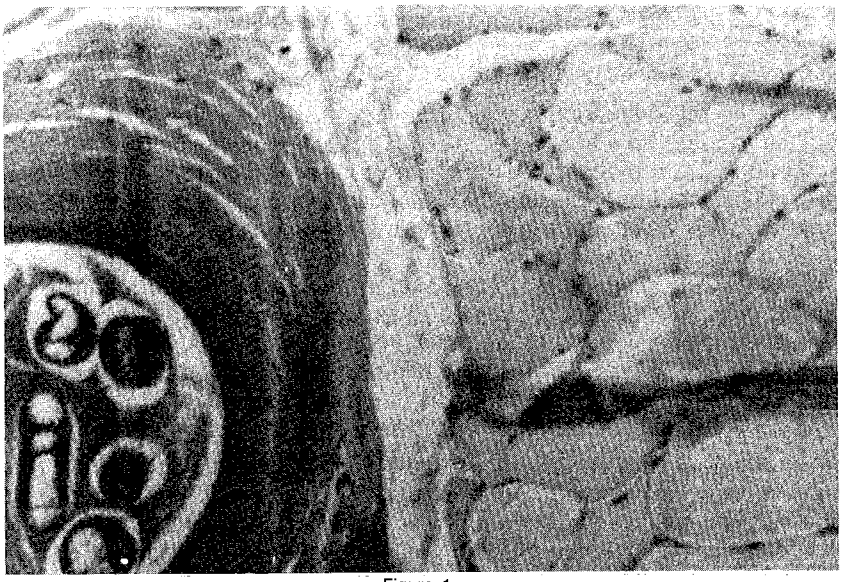

Figure 1.
Cross-section of a lesion of trichinosis in the diaphragm showing thick fibrous capsule of the cyst containing live larva in a homogeneous acellular matrix and the absence of inflammatory cellular infiltrates. PAS staining, × 300 *ca.*

Discussion

An enquiry into the history on the husbandry of the polar bear in question excluded the chances of its acquiring the infection in captivity through food, carrion eating, or cannibalism. The animal attendants have neither found rats in the enclosure of the animal at any time nor have they ever observed any predatory habits. The animal apparently picked up the infection in nature

before its capture at a young age. During its captivity, the polar bear had received mebendazole at 10 mg/kg body weight for two consecutive days, twice a year, for many years as a prophylactic or curative treatment against an intestinal ascarid, *Baylisascaris transfuga*, infection. Mebendazole at this regimen did not show any effect on the muscle larvae of the polar bear although this drug, given as medicated feed to rats infected with *T. spiralis* at 125 to 500 ppm for two weeks, is reported to cause 100 % reduction of live muscle larvae (8).

Presence of viable and infective larvae of *T. nativa* after over twenty years of their encystment in polar bear thus be considered a record and a case of extreme adaptability of the nematode to its host. It is difficult to explain if this extented survival of the muscle larvae is dependent on the host physiology or the nematode species. In other hosts including man infected with *T. spiralis*, the cyst contents start calcifying though this does not necessarily indicate the death of the enclosed larva. Considerable host variation exists with regard to the time required for initiation of calcification; it commences after 60 days in rats, after 80 days in rabbits, after 150 days in pigs, after 115 days in man and after two years in mice (2). Ninety-one per cent of the cystic lesions in the present case showed larvae after over two decades of their encapsulation in the host without appreciable sign of calcification of the cyst contents.

While the rat is an adequate host for *T. spiralis* s.s., other *Trichinella* spp. pools fail to infect this host adequately. The Swiss albino mouse, though not the inbred BALB/c mouse, is uniformally receptive to various *Trichinella* spp. pools (6). As the present isolate has shown adequate infectivity for the Swiss mouse and the Wistar rat appeared nearly refractory to it, this confirms that the rat is not an adequate host for *T. nativa*. The case report of human trichinellosis in Paris in 1985 is attributed to *T. nativa* orginating from a single horse of North American origin (1). Because the present isolate shows an isoenzyme profile identical to the reference *T. nativa* strain (code MURS/NO/84/ISS10) and also shows biochemical identity to the one associated with Paris outbreak, by implication it is conceived that this isolate would be infective for man and horse and thus bears a zoonotic significance.

In agreement with our studies, spindle-shaped gross lesions of *Trichinella* sp. infection of unknown duration in a polar bear, most of which contained viable larvae, are also mentioned (4). However, unlike the present case showing complete absence of inflammatory cellular infiltrates in the lesions, they found few of these cysts surrounded by a small number of lymphocytes and there was almost absence of inflammatory reaction. Inflammatory cellular infiltrates in muscle trichinellosis seem to appear and subside with the age of the infection. In the Swiss albino mice infected with *T. spiralis*, marked leucocytic infiltration of lesions occurs at four weeks of infection, subsides at eight weeks of infection and from 14 weeks onwards, the inflammation continues at a low persistant level (3). The same evolutionary mechanism of cellular infiltrates may be operative in *T. nativa* lesions of polar bear so that over a period of two decades, the cellular infiltration had completely subsided.

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Caractérisation d'une Trichinella isolée d'un ours polaire.

Résumé — Un isolat de *Trichinella*, originaire d'un ours polaire, est étudié par typage isoenzymatique. La souche est identique à *Trichinella nativa*. Bien que les rats Wistar sont presque réfractaires au parasite isolé, les souris Swiss albino sont extrêmement sensibles. Les cystes du diaphragme de l'ours, infesté depuis plus de 20 ans, contiennent encore des larves vivantes, preuve du pouvoir extrême d'adaptation du parasite à son hôte. Les aspects anatomo-pathologiques des lésions sont étudiés ainsi que l'importance zoonotique du parasite isolé.

Typering van een Trichinella-isolaat afkomstig van een ijsbeer.

Samenvatting — Bij autopsie van een ijsbeer werd *Trichinella* geïsoleerd. Na isoenzymatisch onderzoek werd de parasiet getypeerd als *Trichinella nativa*. Terwijl Wistar ratten zo goed als weerstandig bleken te zijn tegenover besmetting, waren Swiss albino muizen zeer gevoelig. Meer dan 20 jaar na de infestatie van de ijsbeer bevatten 91 % van de parasitaire kysten van het diafragma levende larven, wat kan gezien worden als een geval van uitzonderlijke aanpassing van de parasiet aan zijn gastheer. De anatomo-pathologische kenmerken van de letsels worden besproken en er wordt aandacht geschonken aan de zoönotische betekenis van deze vondst.

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REFERENCES

1. de Carneri I, Ancelle T, Dupouy-Camet J, Pozio E: Different aetiological agents cause the European outbreaks of horse-meat induced human trichinellosis. *in* Proceedings of the VII International Conference on Trichinellosis, Alicante, Spain, 2-6 October, 1988 (in press).
2. Gould SE: Anatomic pathology. *in* Trichinosis in Man and Animals (Ed. S.E. Gould). Springfield, Charles C. Thomas, 1970, pp. 147-189.
3. Grove DI, Hamburger J, Weren KS: Kinetics of immunological responses, resistance to reinfection, and pathological reactions to infection with *Trichinella spiralis*. J. Infect. Dis., 1977, **136**, 562-570.
4. McColl KA, Butler R: Trichinosis in a polar bear, *Thalarctos maritimus*, from the Royal Melbourne Zoo. Aust. Vet. J., 1982, **59**, 61.
5. Pozio E: Isoenzymatic typing of 23 *Trichinella* isolates. Trop. Med. Parasit., 1987, **38**, 111-116.
6. Pozio E, La Rosa G, Rossi P: *Trichinella* Reference Centre. Parasitol. Today, 1989, **5**, 169-170.
7. Pozio E, La Rosa G, Rossi P, Murrell KD: New taxonomic contribution to the genus *Trichinella* Railliet, 1895. I. Biochemical identification of seven clusters by gene-enzyme systems. *in* Proceedings of the VII International Conference on Trichinellosis, Alicante, Spain, 2-6 October, 1988 (in press).
8. Thienpont D, Vanparijs OF, Vandesteene R: Anthelmintic and histopathological effects of mebendazole in *Trichinella spiralis* on the rat. *in* Trichinellosis (Ed. C.W. Kim). Stony Brook, N.Y., State University of New York, 1974, pp. 515-527.
9. World Health Organization: Trichinosis, outbreaks associated with horse meat. Wkly Epid. Rec., 1986, **61**, 293-294.