ATTEMPTED EXPERIMENTAL CROSS INFECTIONS WITH MAMMALIAN GUINEA-WORMS, *DRACUNCULUS* SPP. (NEMATODA: DRACUNCULOIDEA)

MARY BEVERLEY-BURTON AND VINCENT F. J. CRICHTON

Reprinted from THE AMERICAN JOURNAL OF TROPICAL MEDICINE AND HYGIENE
Vol. 25, No. 5, September 1976
p. 704–708
Printed in United States of America

THE AMERICAN JOURNAL OF TROPICAL MEDICINE AND HYGIENE
Vol. 25, No. 5

Printed in U.S.A.

ATTEMPTED EXPERIMENTAL CROSS INFECTIONS WITH MAMMALIAN GUINEA-WORMS, *DRACUNCULUS* SPP. (NEMATODA: DRACUNCULOIDEA)

MARY BEVERLEY-BURTON AND VINCENT F. J. CRICHTON*
Department of Zoology, College of Biological Science, University of Guelph, Guelph, Ontario, Canada N1G 2W1

Abstract. *Dracunculus medinensis* and *D. insignis* are morphologically indistinguishable. Experiments to test the susceptibility of various mammalian hosts to these two guinea worm species are described. Infective 3rd-stage larvae of *D. medinensis* were administered to each of four raccoons (*Procyon lotor*): infective 3rd-stage larvae of *D. insignis* were administered to a rhesus monkey (*Macaca mulatta*), two dogs, two ferrets (*Mustela putorius furo*), and a marten (*Martes americana*). *D. medinensis* was not found in the raccoons when necropsies were performed on days 247, 283, 354, and 390 post-infection, respectively. Nine female *D. insignis* containing eggs, embryos, and motile 1st-stage larvae were found in the rhesus monkey 180 days post-infection. Lesions had not formed and the larvae were presumed to be immature and not yet infective as they were comparatively inactive and attempts to infect suitable copepods failed. *D. insignis* was not found in the dogs or the marten, although both ferrets were successfully infected. Variations in susceptibility of various mammalian species to the guinea worm are discussed together with comments on variations in migration routes and sites of emergence in hosts which may be partially refractory. *D. insignis* and *D. medinensis* may represent physiological strains of a single species, or they may in fact be two distinct species which have evolved in different geographical locations.

Human infection with *Dracunculus medinensis* has been recognized for centuries and occurs in many areas of Africa, Asia, and the Middle East. Although human dracunculiasis, presumably introduced by slaves from West Africa, has been observed in the New World (the West Indies and Brazil) it has died out spontaneously in the last 50 years.[1] In North America autochthonous infections reported in the United States have never been confirmed (P. C. Beaver, Tulane School of Public Health and Tropical Medicine, New Orleans, Louisiana. Personal communication, 1973), although guinea worm infection in wild carnivores is widespread in certain areas.[2]

The first report of guinea worm, as such, from a North American carnivore was in 1932 when worms from a fox were identified as *D. medinensis*.[3] Subsequently, conflicting ideas were put forward. Chitwood suggested that the New World wildlife guinea worm was a physiological strain of *D. medinensis* different from that adapted to man in the Old World,[4] while Chandler considered that it was not *D. medinensis* at all but the species originally described as *Filaria insignis* Leidy, 1858.[5] Thus, the combination *Dracunculus insignis* was proposed.

Most authors have used the name *D. insignis* in the increasing number of references to guinea worm from North American wildlife,[2, 6–13] although some[14–16] have persisted in retaining the name *D. medinensis* as the two forms are morphologically indistinguishable.

Thus, the taxonomy of guinea worm parasitizing mammals remains confused and, although the situation in North America has been partially clarified,[13] the relationship between *D. medinensis* and *D. insignis* has yet to be resolved.

This paper reports experiments to test the susceptibility of various mammalian hosts to these two guinea worm species. Attempts were made to transmit *D. medinensis* to raccoons (*Procyon lotor*) and *D. insignis* to a rhesus monkey (*Macaca mulatta*), dogs, ferrets (*Mustela putorius furo*) and a marten (*Martes americana*).

Accepted 30 January 1976.

* Present address: Manitoba Department of Mines, Resources and Environmental Management, 1-139 Tuxedo Boulevard, Box 12, Winnipeg, Manitoba, Canada R3N 0H6.

MATERIALS AND METHODS

Attempted transmission of D. medinensis *to raccoons*

Copepods (*Cyclops* sp.) containing infective 3rd-stage larvae of *D. medinensis* were received from Dr. R. Muller of the London School of Hygiene and Tropical Medicine, London, England. Initially the 1st-stage larvae were obtained from an Indian who had recently arrived in England. Infection of copepods with guinea worm larvae and their subsequent maintenance have been described by Muller.[1] On arrival at Guelph, the larvae were processed using routine methods[17] and approximately 100 were administered by stomach tube (#9) to each of four ether-anesthetized raccoon kits (2 males, 2 females).* The 3-month-old kits were born and raised in captivity and had been fed commercial frozen mink feed. A female sibling was used as an uninfected control. The hair on the lower limbs of these animals was removed 121 days after administration of infective larvae to facilitate attempts to locate female worms by palpation on days 121, 151, 181, 195, 209, 223, 237, 251, 265, 279, 293, 307, and 335. Necropsies were performed on days 247, 283, 354, and 390. The control was killed and examined on day 396.

Attempted transmission of D. insignis *to a rhesus monkey*

Dracunculus insignis, obtained originally from a wild-caught raccoon taken in early summer, was cycled experimentally in a ranch-bred mink (*Mustela vison*). First-stage larvae were obtained from a female guinea worm dissected from the leg of this mink 340 days post-infection. Approximately 250 *Cyclops vernalis* were exposed to these larvae, using previously described techniques,[17] and maintained at 23 to 24° C. After 18 days, infective 3rd-stage larvae were collected[17] and approximately 400 were administered by stomach tube (#9) to a male rhesus monkey which was sedated with Sernylan. The monkey, estimated to be 3.5 years old, had been held at the Connaught Medical Research Laboratories in Toronto for 6 months prior to administration of the infective larvae. It was part of a colony imported from India and other rhesus monkeys in the group served as uninfected controls.

Necropsy was carried out 180 days after administration of infective larvae.

Attempted transmission of D. insignis *to dogs*

First-stage larvae were obtained from a female worm removed from a naturally infected, road-killed raccoon and used to infect approximately 500 *C. vernalis* as described previously. Approximately 250 infective 3rd-stage larvae were administered in a small amount of canned dog food to an 8-week-old Collie/Shepherd cross. A sibling served as an uninfected control. Necropsy was performed on both dogs 365 days after administration of infective larvae. Another dog received 210 larvae by intubation and was examined by palpation daily up to 390 days post-infection. On both occasions raccoons* were infected and served as controls.

Attempted transmission of D. insignis *to ferrets and a marten*

First-stage larvae, obtained from a raccoon, were used to infect *C. vernalis* as described previously. Approximately 220 infective 3rd-stage larvae were administered by intubation to each of two ferrets,* and necropsies were performed 244 and 245 days later. A marten* received 160 larvae. In both instances, raccoons* were infected and served as controls.

RESULTS

Dracunculus medinensis was not recovered from any of the four raccoons given infective larvae, nor was there any trace of an infection.

Evidence of migrating *D. insignis* females in the rhesus monkey was not observed prior to necropsy at 180 days post-infection. At necropsy 9 female worms containing eggs, embryos and motile 1st-stage larvae were recovered: 2 (measuring 224 and 199 mm) in the right tarsal region and 3 in the left tarsal region; 1 coiled on the inner side of the left upper arm, 1 in the left lower arm, and 1 in each of the right and left carpal regions. All worms were lying in subcutaneous connective tissue and no obvious host

* Food was withheld from all experimental animals for 12 h prior to attempted transmission and for 12 h afterwards.

* Born and raised in captivity.

reaction was seen. No sign of lesion development in the vicinity of the worms was observed. A single male worm was found in connective tissue under the left latissimus dorsi muscle. Neither guinea worms nor signs of them have ever been observed in the rhesus monkeys imported into the Connaught Medical Research Laboratories. Ten 1st-stage larvae taken from each of 3 larvigerous females measured 621 (589–648) μ long. Selected motile 1st-stage larvae from these females failed to develop in 250 *C. vernalis* exposed routinely and maintained at 23 to 24° C for 21 days.

D. insignis was not observed in either of the dogs given infective larvae or in the dog which served as a control. Both raccoons used as controls developed patent infections.

Guinea worms were found in the two ferrets experimentally infected with *D. insignis*. One ferret carried a 4th-stage female larva (21 mm long) which was located in the subcutaneous tissue anterior to the dorsal region of the base of the tail. The other ferret harbored one female in the subcutaneous tissue of the inguinal region. In addition, a dead partially resorbed female was found in the subcutaneous tissue of the tarsal region: it had not passed its larvae. No worms were recovered from the marten although both raccoons used as controls developed patent infections.

DISCUSSION

In isolation, the experimental infection of a rhesus monkey with *D. insignis* would appear to support the theory that *D. medinensis* and *D. insignis* represent physiological strains of a single species.[4, 18] However, the subsequent finding that *D. medinensis* could not be transmitted to 4 raccoons suggests that they may be two distinct forms.

D. insignis has been considered primarily a parasite of raccoons and secondarily of mink,[2] and experimental data indicate that mink may, in fact, be somewhat refractory to infection as guinea worms were recovered from 86.8% of 38 attempted transmissions to raccoons at a recovery rate of 4.3%, while for mink the levels were 58.1% of 31 attempts and 1.1%, respectively.[17] Dogs may also be refractory to *D. insignis* as transmission attempts failed in the present study and the number of recorded cases of guinea worm in this host in North America is only ten.[19] This is in contrast to *D. medinensis* which has frequently been reported from naturally infected dogs,[1] and 27 of 42 dogs (64.3%) were successfully infected experimentally by feeding each with only 25 to 30 *D. medinensis* larvae in cyclops.[20] Other North American mammals may prove completely resistant to *D. insignis* as 139 martens (*Martes americana*), 3 long-tailed weasels (*Mustela freneta*), 6 timber wolves (*Canis lupus*), 5 red foxes (*Vulpes vulpes*), 7 lynx (*Lynx canadensis*), and 6 beavers (*Castor canadensis*), taken in the trapping seasons of 1969 to 1972, were examined and found to be negative for guinea worm.[2]*

In addition, an attempt to infect a marten failed in the present study. Similarly, man may be completely resistant to *D. insignis* as there are, as yet, no authentic records of autochthonous infections in North America. In one case[21] the "worm" may not have been a helminth, certainly not *Dracunculus* sp. if the dimensions are accurate, and another case reported by Michelson in 1969[1] was later recognized as a *Dermatobia* lesion (P. C. Beaver, personal communication). It is of interest that in parts of the southern United States where a high prevalence of *D. insignis* in raccoons occurs, at least in some years, and human sparganosis is not uncommon, dracunculiasis is unknown (P. C. Beaver, 1973, personal communication).

In experimentally infected rhesus monkeys, female *D. medinensis* utilize a subcutaneous migration route over the ventral trunk region with conspicuous sinuosities appearing on the abdomen and thorax 8 months after infection (i.e., 2–4 months before emergence).[1] Migrating guinea worms have never been found in any rhesus monkeys imported by the Connaught Research Laboratories where the majority of animals are maintained for more than 6 months and many are kept for several years. This strongly suggests that rhesus monkeys from India are unlikely to carry natural infections of *D. medinensis* especially as the only reference to guinea worm from the rhesus monkey is an undated deposition by

* Although the appearance of lesions in the extremities, due to the emergence of larvigerous female guinea worms, is seasonal (spring and summer) data concerning the prevalence and intensity of infections can be collected when animals are taken during the trapping season (late fall and winter) if the entire carcass is examined for males and non-larvigerous females.[2, 13]

an unknown collector in the London School of Hygiene.[1]

It is of interest that female *D. insignis* in the rhesus monkey did not follow the superficial subcutaneous migration route over the trunk described for *D. medinensis* in this host. It is possible that atypical migration routes and emergence sites are seen more frequently in hosts which are to some degree refractory. Examples of emergent, and non-emergent, larvigerous *D. medinensis* in man in unusual sites have been reported,[1] but their occurrence away from the limbs is rare. In contrast the only muskrat (*Ondatra zibethicus*) (of 105 examined) found to be infected with *D. insignis* harbored one larvigerous female coiled on the mesentery in the abdominal cavity and another at the base of the tail, and an infected opossum had a single larvigerous female coiled around one testicle.[22] Four female *D. insignis* found in a dog in Canada were removed from the flanks, neck, and intercostal region. One, on the left flank, had produced a lesion and at least two others were larvigerous.[19]

The 1st-stage *D. insignis* larvae taken from non-emergent larvigerous females in the rhesus monkey at 180 days post-infection were not yet infective although their body length (621 μ, mean-range 589–648 μ) was similar to that of larvae from patent infections in raccoons (665 μ, mean-range 596–857 μ) and mink (698 μ, mean-range 673–749 μ).[13] Also, the larvae contained in females removed from a rhesus monkey moved sluggishly compared to the frantic activity of larvae removed from mature females in typical hosts. It is probable that 180 days is insufficient time for the production of fully developed, infective larvae in spite of the fact that the females were mature and similar in size to worms of known age in raccoons and mink.[17] However, the size and location of female worms at any particular time probably depends more on the number of days since fertilization rather than on the number of days post-infection. For example, female *D. insignis* have been detected, by palpation, in the tarsal region of experimentally infected mink only 120 days post-infection,[17] indicating that those particular worms were fertilized soon after entry into the final host and immediately migrated to the extremities. Although it was presumed that these worms were larvigerous it is unlikely that the larvae were infective.

In summary, we are still faced with two possibilities regarding the status of *D. insignis* and *D. medinensis*: either they represent physiological strains of a single species or they are two distinct species which have evolved in different geographic locations.

ACKNOWLEDGMENTS

We thank Dr. R. Ko, University of Hong Kong, Dr. M. Walcroft, Connaught Medical Research Laboratories, Toronto and Dr. L. Uhazy, University of Guelph for their assistance. Financial support was provided, in part, by the N.R.C. of Canada (Grant #575-91).

REFERENCES

1. Muller, R., 1971. *Dracunculus* and dracunculiasis. *Adv. Parasitol., 9:* 73–151.
2. Crichton, V. F. J., and Beverley-Burton, M., 1974. Distribution and prevalence of *Dracunculus* spp. (Nematoda: Dracunculoidea) in mammals in Ontario. *Can. J. Zool., 52:* 163–167.
3. Benbrook, E. A., 1932. *Dracunculus medinensis* (Linnaeus, 1758) appears in the United States as a parasite of the fox. *J. Am. Vet. Med. Assoc., 81:* 821–824.
4. Chitwood, B. G., 1933. Does the guinea-worm occur in North America? *J. Am. Med. Assoc., 100:* 802–804.
5. Chandler, A. C., 1942. The guinea-worm *Dracunculus insignis* (Leidy, 1858), a common parasite of raccoons in east Texas. *Am. J. Trop. Med., 22:* 153–157.
6. Cheatum, E. L., and Cook, A. H., 1948. On the occurrence of the North American guinea worm in mink, otter, raccoon and skunk in New York State. *Cornell Vet., 38:* 421–423.
7. Chitwood, B. G., 1950. The male of *Dracunculus insignis* (Leidy, 1858) Chandler, 1942. *Proc. Helminthol. Soc. Wash., 17:* 14–15.
8. Crites, J. L., 1963. Dracontiasis in Ohio carnivores and reptiles with a discussion of the dracunculid taxonomic problem (Nematoda: Dracunculidae). *Ohio J. Sci., 63:* 1–6.
9. Fyvie, A., 1964. *Manual of Common Parasites, Diseases and Anomalies of Wildlife in Ontario,* 1st ed. Ontario Department of Lands and Forests.
10. Fyvie, A., 1966. A review and current survey of the common diseases and parasites of wildlife in Ontario. Ontario Department of Lands and Forests, *Section Report (Wildlife) No. 63.*
11. Webster, W. A., and Casey, D. A., 1970. The occurrence of *Dracunculus insignis* (Leidy, 1858) Chandler, 1942 in a skunk from Ontario, Canada. *J. Wildl. Dis., 6:* 71.
12. Gibson, G. G., and McKiel, D. A., 1972. *Dra-*

cunculus insignis (Leidy, 1858) and larval *Eustrongylides* sp. in a muskrat from Ontario, Canada. *Can. J. Zool., 50:* 897–901.

13. Crichton, V. F. J., and Beverley-Burton, M., 1973. *Dracunculus lutrae* n. sp. (Nematoda: Dracunculoidea) from the otter, *Lutra canadensis*, in Ontario, Canada. *Can. J. Zool., 51:* 521–529.
14. Erickson, A. B., 1946. Incidence of worm parasites in Minnesota Mustelidae and host lists and keys to North American species. *Am. Midl. Nat., 36:* 494–509.
15. Mirza, M. B., 1957. On *Dracunculus* Reichard, 1759, and its species. *Z. Parasitenk., 18:* 44–47.
16. Medway, W., and Soulsby, E. J. L., 1966. A probable case of *Dracunculus medinensis* (Linn., 1758) Gallandant, 1773, in a dog in Pennsylvania. *J. Am. Vet. Med. Assoc., 149:* 176.
17. Crichton, V. F. J., and Beverley-Burton, M., 1973. Migration, growth and morphogenesis of *Dracunculus insignis* (Nematoda: Dracunculoidea). *Can. J. Zool., 53:* 105–113.
18. Crichton, V. F. J., and Beverley-Burton, M., 1973. Identification of guinea-worm species. *Trans. R. Soc. Trop. Med. Hyg., 67:* 152.
19. DeRoth, L., and Frechette, J.-Ls., 1975. *Dracunculus insignis* (Leidy, 1858) chez un Berger Allemand. *Can. Vet. J., 16:* 176–178.
20. Issajev, L. M., 1934. Experimental dracunculiasis in the dog. *Med. Parazytol., 3:* 231–238.
21. Spiers, R. E., and Baum, A. H., 1953. Dracunculosis (autochthonous in U.S.A.). *J. Kansas Med. Soc., 54:* 553–554.
22. Crichton, V. F. J., 1972. The biology of *Dracunculus* spp. (Dracunculoidea: Dracunculidae) in wildlife from Ontario. *Ph.D. Thesis, University of Guelph, Ontario.*