***HALICEPHALOBUS GINGIVALIS* (STEFANSKI, 1954) FROM A FATAL INFECTION IN A HORSE IN ONTARIO, CANADA**

with comments

on the validity of *H. deletrix* and a review of the genus

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Summary :

Halicephalobus gingivalis (Stefanski, 1954), from a fatal infection in a horse in Ontario, Canada, was cultured and restudied. Although the original description given by Stefanski (1954) was satisfactory, Anderson & Bemrick (1965), in describing *H. deletrix* (= *Micronema deletrix*), claimed Stefanski's description was « inadequate » and the species a « species inquirenda ». Thus, infections in horses and humans have been assigned to *H. deletrix*. We believe the species reported in horses and humans is *H. gingivalis* and that *H. deletrix* is its synonym. *H. gingivalis* is separated herein from forms found free-living. The genital tract in the advanced fourth stage of *H. gingivalis* is didelphic and amphidelphic and terminal ends of the horns are reflected, the anterior one ventrally, the posterior one dorsally. In the adult parthenogen the latter forms a short ovary, whereas most of the anterior horn forms a combined uterus-oviduct as a receptacle for a single large egg which is laid in the 2-cell or multi-cell stage. Eggs in the 2-cell stage embryonated to larvae in 17 hours at 28 °C but did not hatch until an additional 24 hours. First-stage larvae were unusually large and variable in length (136-199 µm $x = 168$). Inactive third-stage larvae were 180-240 µm ($x = 203$) in length. The possible route of infection in horses and humans is briefly discussed.

KEY WORDS : *Halicephalobus gingivalis*, *Micronema*, Nematoda, Panagrolaimidae, horse, taxonomy.

Résumé : *HALICEPHALOBUS GINGIVALIS* (STEFANSKI, 1954) DANS UNE INFECTION FATALE CHEZ UN CHEVAL D'ONTARIO, CANADA AVEC COMMENTAIRE SUR LA VALIDITÉ DE *H. DELETRIX* ET UNE REVUE DU GENRE

Une souche d'*Halicephalobus gingivalis* (Stefanski, 1954), isolé d'une infection fatale d'un cheval d'Ontario au Canada, a été cultivée et réétudiée. Bien que la description originale de Stefanski (1954) soit satisfaisante, Anderson & Bemrick (1965), dans leur description de *H. deletrix* (= *Micronema deletrix*), ont considéré que la description de Stefanski était « inadéquate » et que l'espèce devrait être considérée comme « species inquirenda ». Ainsi, les infections chez le cheval et l'humain ont été attribuées à *H. deletrix*. Nous croyons que l'espèce rapportée chez le cheval et l'humain est *H. gingivalis* et que *H. deletrix* est son synonyme. Dans l'étude présente, *H. gingivalis* est isolé de formes trouvées libres. La branche génitale du quatrième stade avancé de *H. gingivalis* est didelphique et amphidelphique et les extrémités des cornes sont repliées, l'antérieure du côté ventral, et la postérieure du côté dorsal. Chez l'adulte parthogénétique, cette dernière forme un petit ovaire, tandis que la majeure partie de la corne antérieure forme un utérus-oviducte réunis en un réceptacle pour un gros œuf unique qui est pondue au stade bicellulaire ou multi-cellulaire. Les œufs du stade bicellulaire se sont développés en larves en 17 h à 28 °C mais n'ont éclos que 24 h plus tard. Les larves du premier stade étaient anormalement grosses et variables en longueur (136-199 µm $x = 168$). Les larves inactives du troisième stade mesuraient 180-240 µm ($x = 203$). La voie probable d'infection chez le cheval et l'humain est discutée brièvement.

MOTS CLÉS : *Halicephalobus gingivalis*, *Micronema*, Nematoda, Panagrolaimidae, cheval, taxonomy.

INTRODUCTION

Halicephalobus gingivalis was first described accurately by Stefanski (1954) on the basis of worms found in a granuloma in the gingivae of a horse in Poland. He placed the species in the genus *Rhabditis*. It was transferred to the genus *Tricephalobus* by Dougherty (1955), a decision reproduced without comment by Baker (1962) and Levine

(1968). Sudhaus (1976) placed *gingivalis* in *Trilabiatius* and later Andrassy (1984) placed the species in *Halicephalobus* Timm, 1956 where it remains today. Although *H. gingivalis* was reported in a horse and has priority, reports of similar infections in horses and humans have almost invariably ignored this species and identified the agent as *Micronema deletrix* or, more recently, *Halicephalobus deletrix*. *Micronema deletrix* from a « nasal tumour » of a horse in Minnesota, USA was described by Anderson & Bemrick (1965); the clinical pathology of this case was described by Johnson & Johnson (1966) who reported the nematodes mainly in large granulomas in the maxillae. Anderson & Bemrick (1965) stated in a short postscript to their paper « *Rhabditis gingivalis* Stefanski (1954) described from a granuloma of the gum of a horse appears to be a species of *Micronema* but is inadequately described

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and must be considered *species inquirenda* ». Thus it came about that subsequent reports of these kinds of nematodes, causing serious and generally fatal infections in horses and humans, ignored Stefanski's species and were invariably assigned to *Micronema deletrix* or later *Halicephalobus deletrix*. Blunden *et al.* (1987) placed a further cloud over *gingivalis* when they stated in references to « Stefanski (1953) » « ... nematode not described ... ».

Andrassy (1984) recognized the name *Micronema* was preoccupied and he transferred the species of *Micronema* to the genus *Halicephalobus* Timm, 1956. He placed *gingivalis* in this genus. Andrassy (1984) and Geraert *et al.* (1988) in reviews of the genus *Halicephalobus* apparently regarded *H. deletrix* as a possible synonym of *H. gingivalis*. Although they gave no reasons for this possibility it can probably be assumed that it was because *H. gingivalis* and *H. deletrix* were both from lesions in horses.

We have recently collected nematodes from a fatal infection in a horse in Ontario that agree in all particulars with Stefanski's species. In addition, we have successfully cultured the nematode for the first time and studied its development and are able to provide a new and more detailed description based on abundant living and fixed material. We establish *H. gingivalis* as a valid species of *Halicephalobus* and confirm that *H. deletrix* is a synonym. In addition, we consider briefly the possible relationship of *H. gingivalis* to the known free-living members of *Halicephalobus*.

MATERIALS & METHODS

In December 1997 an 18-year old horse was admitted at the Ontario Veterinary College with a fatal infection of *H. gingivalis*¹. Nematodes were collected from lesions within the mandible at post mortem by maceration of tissue in saline. Parasites were then inoculated onto Luria broth agar containing 50 µg/ml ampicillin onto which ampicillin-resistant *Escherichia coli* were also plated, and maintained on the bench at 18-22 °C. Active proliferation, with generation of all life-cycle stages occurred on this medium. Parasites from these plates were then established as actively proliferating populations in saline containing *E. coli*, solute of sterilized bovine faeces and 4 mm³ pieces of agar, as this culture system produced larger numbers of parasites than the solid agar system.

Live nematodes were studied in saline under coverslips sealed with vaseline. The adults remained active for prolonged periods and laid eggs. The eggs readily

embryonated and hatched and the larvae grew for several days. It was possible to note the rate of development and hatching under these conditions.

Some adult nematodes were placed on a glass slide in a drop of saline and subjected to mild heat which immobilized them without affecting their anatomy. The drop of saline with the relaxed nematodes was covered with a coverslip sealed with vaseline and major morphological measurements were made. Droplets of lipid in the intestinal cells of the nematodes tended to obscure the morphology of parts of the reproductive system. To solve the problem some nematodes were fixed in hot glycerine alcohol (70 % ethanol and 5 % glycerine) and the mixture was exposed to air for a day or two to allow the alcohol to evaporate. The nematodes were then examined in pure glycerine. The process removed lipid and helped expose the details of the reproductive system. Some nematodes were immersed in a solution of aniline blue which differentially stained the ovary in the mature worms and the genital primordia in third and fourth-stage nematodes.

RESULTS

HALICEPHALOBUS GINGIVALIS (STEFANSKI, 1954) (Fig. 1-8)

Synonyms

Rhabditis gingivalis Stefanski, 1954;
Tricephalobus gingivalis (Stefanski, 1954) Dougherty, 1955;
Halicephalobus gingivalis (Stefanski, 1954) Andrassy, 1984;
Trilabiatius gingivalis (Stefanski, 1954) Sudhaus, 1976;
Micronema deletrix Anderson & Bemrick, 1965;
Halicephalobus deletrix (Anderson & Bemrick, 1965); Andrassy, 1984.

General

Nematoda, Rhabditoidea, Panagrolaimidae Thorne 1937, Tricephalobinae Andrassy, 1976. Small parthenogenetic nematodes. Oesophagus with median swelling.

Parthenogenetic Female (For morphometrics see Table I). Body curved slightly ventrally when fixed (Fig. 8). Six prominent but transparent, rounded lips (Fig. 4). Cuticle smooth and thin with extremely obscure transverse striations in anterior region. Tail conical, ending in fine point, phasmids short distance behind anus (Fig. 7). Buccal cavity elongate, divided into two sections with ring at base (Fig. 4). Nerve ring between valved bulb and medium swelling of oesophagus (Fig. 8). Excretory pore adjacent to nerve ring. Intestinal cells packed with round lipid droplets along

1. The clinical account of this case will be published elsewhere.

Figs. 1-8. – *Halicephalobus gingivalis* (Stefanski, 1954).

Fig. 1-3. – Development of the eggs from the two-celled stage to larva.

Fig. 4. – Cephalic end showing rounded, transparent lips and buccal cavity.

Fig. 5. – Genital primordium of the third-stage larva.

Fig. 6. – Genital system in the fourth stage larva (didelphic, amphidelphic).

Fig. 7. – Caudal end of parthenogen showing anus and phasmids.

Fig. 8. – Gravid parthenogen (lateral view) with fully developed but unsegmented egg in modified anterior horn of reproductive tract and short reflexed ovary of the posterior horn. Lipid droplets abundant in intestinal cells.

Author(s)	Stefanski (1954)	Guelph (present)	Anderson & Bemrick (1965)
N	?	10	? 28
Method	From tissue fixed in formalin	Living, immobilized with mild heat	From frozen tissue
Length body	250-430	311-411 (367)	250
<i>a</i>	17-23	15-20 (18)	15-20 (17)
<i>b</i>	3.5-3.8	3.2-4.5 (3.9)	2.9-3.6 (3.3)
<i>c</i>	5.5-7.0	4.5-6.6 (5.5)	4.4-6.3 (5.1)
<i>v</i>	60-65	46-61 (56)	56-63 (59)
Maximum width	14-20	18-21 (20)	—
Length Buccal cavity	? 4-5**	8-11 (10)	—
Length Oesophagus	70-90	76-90 (84)	—
Vulva (from ant. End)	90-210	155-255 (203)	—
Length Tail	40-60	59-75 (66)	—
Size of Eggs	40 × 20	48-51 × 16-21	32-46 × 9-11

* The De Man ratios (*a* to *c* + *v*) favoured by plant and soil nematologists are as follows: *a* = body length/maximum width; *b* = body length/length of oesophagus; *c* = body length/tail length; *v* = distance of vulva from anterior end as percent of total length.

Other measurements are in μm .

** Stefanski's figures for the length of the buccal capsule are obviously in error as his illustration shows the figures should be about 10 μm .

Table I. – Comparative Measurements of (adults) *Halicephalobus gingivalis* and *H. delectrix* from Horses*.

entire length. Vulva a broad lateral slit on slight elevation. Reproductive system clearly didelphic and amphidelphic in fourth-stage larva (Fig. 6). In fully developed adult posterior arm of reproductive tract reduced to ovary only with about 9-12 oocytes increasing in size from posterior end to anterior region (Fig. 8). Terminal end of ovary curved ventrally. In fully gravid nematode anterior arm of reproductive tract modified as combined oviduct and uterus containing single large, elongate egg (16-21 × 48-51 μm in size) with thin pliable shell. Terminal end of anterior arm finger-like, non-functional and bent dorsally and posteriorly. Egg unusually large (Figs. 1-3), segmented or unsegmented *in situ* but generally deposited by female in 2-cell or 8-cell stage, but larvating and hatching in moribund parent (matricidal endotoky). Egg compressed laterally when being expressed from parent. Eggs embryonating from 2-cell stage to larva in 17-18 hours at 28 °C and 24 hours at 24-25 °C (Fig. 1-3). Eggs hatching about 24 hours after larvae formed in egg. Newly hatched larvae unusually long, 136-199 ($x = 168$) μm in length and 9-10 ($x = 9.6$) μm in width ($N = 10$). In unsuitable conditions larvae cease to develop and become quiescent at third-larval stage 180-240 ($x = 203$) μm in length and 9-10 ($x = 9.5$) μm in width ($N = 10$).

Cultured nematodes were identical in morphology to those found in the lesions from the horse.

Host and Locality: Horse (*Equus caballus* L.), Ontario, Canada.

Location in Host: Gums (*gingivae*) mandible (marrow) and brain.

Specimens: United States National Parasite Collection No. 87837.

DISCUSSION

Morphometric data indicate that the specimens described by Stefanski (1954) and those described herein are conspecific (Table I). In addition, Stefanski's illustration accords with the new observations, including the structure of the reproductive system which he noted was amphidelphic.

Unfortunately the type specimens of *H. delectrix* cannot be found and may no longer exist. In describing *H. delectrix*, Anderson & Bemrick (1965) apparently measured 28 individuals but they gave the length of the body as 0.250 mm (without ranges) which is smaller than the specimens studied herein but is accommodated by the original description of *H. gingivalis*. We assume Anderson & Bemrick (1965) measured smaller adult worms which were not yet fully grown. These types of adults were predominant in our cultures but not included in our data in favour of larger adults with fully-shelled eggs. Presumably Stefanski (1954) included some smaller adults in his data as well as he reported a range of 250-430 μm which covers not only our specimens but those of Anderson & Bemrick (1965).

Anderson & Bemrick (1965) gave the width of the eggs as only 9-11 μm . These smaller figures can be explained if one assumes eggs were measured only *in utero* and were not developed to the extent that the shell had formed (see Fig. 1 in Anderson & Bemrick, 1965). In the present study eggs were measured after they were expelled. Even eggs with shells can be compressed *in utero* as the shell is markedly flexible. Fine cuticular « annulations » were reported in the cuticle of *H. delectrix* by Anderson & Bemrick (1965). Such « annulations » have never been reported in any other description of *Halicephalobus* spp. although extremely fine striations can be seen in the anterior region in our material. It is possible that the specimens used by Anderson & Bemrick (1965) were somewhat contracted by freezing since the worms were removed from frozen tissue. Very fine striations are exceedingly difficult to depict in illustrations and there is no doubt the authors have overemphasized them in their figure. All other features, including the illustration of the reproductive system agree completely with *H. gingivalis* and we regard *H. delectrix* as a synonym of this species.

In addition to *H. gingivalis* and *H. deletrix* which were originally described from specimens found in horses, seven other species have been described in various situations as follows:

- (1) *H. limuli* Timm, 1956 (the genotype) found at the mouth of a freshwater stream in Pakistan.
- (2) *H. similigaster* (Andrassy, 1952) found in dark brown water in the trunk of a tree in Germany.
- (3) *H. minutum* (Körner, 1954) found in moist mulch in the trunks of spruce and sycamore trees in Germany.
- (4) *H. parvum* (Körner, 1954) found in moist mulch in the trunk of oak and linden trees in Germany.
- (5) *H. palmaris* (Lordello & Oliveira, 1963) found in the stem of a plant *Roystonea oleracea* in Brazil.
- (6) *H. intermedia* (Pokrovskaja, 1964) found in galls on cucumber roots in Russia.
- (7) *H. laticauda* Geraert, Sudhaus, Lenaerts and Bosmans, 1988 found in a water pit in a coal mine in Belgium.

The De Man measurements (for definitions see Table I) are not helpful in distinguishing species of *Halicephalobus* because there is so much overlap in the data between species (Table II). The shape of the tail and descriptions of the reproductive tract seem to distinguish *H. gingivalis* from other species in the genus. The reproductive tract of *H. limuli* is said to be prodelphic and monodelphic unlike that in *H. gingivalis*. *H. minutum* and *H. parvum* have much more slender tails than that in *H. gingivalis*. In *H. intermedia* the reproductive tract is shown to be didelphic and amphidelphic but the specimen illustrated was apparently not gravid and the reproductive tract is similar to that in the fourth-stage larvae of *H. gingivalis*; the presence of this species in galls on the roots of cucumbers suggests it must be distinct from *H. gingivalis*. *H. palmaris* is described as monodelphic, unlike *H. gingivalis*. *H. laticauda* resembles *H. gingivalis* but the cephalic end is wider and the authors refer to "... inner sclerotizations at the tip ..." of the tail. The reproductive tract has not been properly described.

There have been three reports of *Halicephalobus* (= *Micronema*) infections in humans, all in North America and fatal. In one case in Canada, the nema-

todes presumably entered lacerations contaminated with manure (Hoogstraten & Young, 1975) and in another case (in the United States) the route of entry was conjectured to be decubitus ulcers on the buttocks (Gardiner *et al.*, 1980). In the third case, also in the United States, no obvious lesion was observed that would have explained entry (Shaddock *et al.*, 1979). Some 25 cases of infection have been reported in horses and they have a wide geographic distribution as follows:

Poland - (Stefanski, 1954);
 United Kingdom - (Angus *et al.*, 1992; Blunden *et al.*, 1987; Khalil *et al.*, 1979);
 Netherlands - (Keg *et al.*, 1984, Linde-Sipman & Gruys, 1970);
 Switzerland - (Pohlenz *et al.*, 1981);
 Columbia - (Payan *et al.*, 1979);
 Egypt (Ferris *et al.*, 1972);
 Japan (Yoshihara *et al.*, 1985);
 North America - (Alstad & Berg, 1979; Cho, 1985; Darien *et al.*, 1988; Dunn *et al.*, 1993; Johnson & Johnson, 1966; Jordan *et al.*, 1975; Kreuder *et al.*, 1996; Pletcher & Howerth, 1980; Powers & Benz, 1977; Rames *et al.*, 1995; Rubin & Woodward, 1974; Ruggles *et al.*, 1993; Simpson, 1993; Simpson *et al.*, 1988; Spalding *et al.*, 1990; Stone *et al.*, 1970; Trostle *et al.*, 1993).
Halicephalobus gingivalis seems to be a cosmopolitan species and one wonders if it might have a predilection to develop in equine manure and this might account for its rather frequent appearance in horses. The route of entry into the horse may be varied. It can probably develop in plant material with bacteria embedded in the gums and between the teeth especially in older animals. This would account for reports involving the maxillae and mandibles. The worms may eventually get into the blood and be disseminated to other regions of the body, including, most importantly, the central nervous system (Ruggles *et al.*, 1993).

Dunn *et al.* (1993) & Payan *et al.* (1979) have reported invasions of the prepuce and one might expect a soil nematode would have the opportunity to invade this region of a horse. Simpson *et al.* (1988) reported *H. deletrix* in granulomas in the leg as well as in other regions of the body. Such infections might be initiated by nematodes invading a lesion in the skin. Rames *et al.* (1995) reported an infection which started in the orbit and progressed to the brain. Orbital infections could be initiated by larvae of *H. gingivalis* carried on the body of muscoid flies like *Musca autumnalis*, the face fly introduced in the 1950's to North America and now a widespread pest of horses and cattle.

In making diagnoses it is important to study the nematodes carefully. Grenier (1991) found a free-living nematode causing mastitis in a mare that could not be assigned to *Halicephalobus*. A review of the literature

Species	Length	a	b	c	v
<i>gingivalis</i> *	311-411 (367)	15-20	3.2-4.5	4.6-6.6	46-61
<i>limuli</i>	426-460	20.0-21.3	4.0-4.8	6.7-7.0	59.3-61.2
<i>similigaster</i>	235-385 (380)	17.3-21.0	3.7-4.3	4.0-4.6	53.6-55.8
<i>minutum</i>	266-283	22.2-24.8	3.9-4.3	5.2-6.5	57.4-63.1
<i>parvum</i>	251-409	17.0-24.2	4.0-5.2	3.8-4.5	53.3-55.8
<i>palmaris</i>	361-410	16.8-18.6	3.8-4.5	6.9-7.5	56.6-60.2
<i>intermedia</i>	230-324	12.7-22.6	3.2-4.5	5.0-6.8	58.2-65.8
<i>laticauda</i>	255-347	24-29	3.3-4.2	4.5-7.3	4.1-6.2

Table II. - De Man's ratios of the various species of *Halicephalobus* (for explanation see Table I).

suggests most infections have been diagnosed correctly but the possibility of other free-living nematodes being involved cannot be ruled out.

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