Vietnam Journal of Biotechnology **21**(2): 327-336, 2023

THE ABUNDANCE OF PARASITIC NEMATODES *HALICEPHALOBUS* SPECIES (NEMATODA: RHABDITIDA) INVADING HUMANS AND ANIMALS IN NATIONAL PARKS OF VIETNAM

Le Tho Son✉**, Bui Thi Mai Huong, Ha Bich Hong, Nguyen Thi Thu**

Vietnam National University of Forestry, Xuan Mai Town, Chuong My District, Hanoi, Vietnam

✉To whom correspondence should be addressed. E-mail: sonlt@vnuf.edu.vn

Received: 22.5.2023
Accepted: 28.6.2023

SUMMARY

Halicephalobus nematodes are parasites in horses, ruminants, and humans. They with the most demonstrated *H. gingivalis* infect the hosts and cause granulomas to fatalities. Anthelmintic treatments are not effective in many cases, so precautions against *Halicephalobus* infection based on the understanding of life history may help. So far, ten species have been globally identified. We isolated many wild-type *Halicephalobus* sp. strains in vegetation samples collected from two national parks (Cat Tien and Cuc Phuong National Parks) in Vietnam and cultured them on bacteria. Thus, *Halicephalobus* nematodes could live in the wild and feed on bacteria. In previous studies, horses were predominantly found to be hosts of *Halicephalobus* sp. nematodes, but in this study they are not presented in the parks, suggesting that other wild animals such as ruminants in the parks should be their hosts. In addition, we analyzed the 18S rDNA sequences of the isolated nematodes and found that they are likely divergent between the two ecological systems, suggesting the two nematode communities have evolved differently.

Keywords: soil-living nematode, helminthiasis, parasitic nematodes, DNA barcoding

INTRODUCTION

Ten species of the nematode genus *Halicephalobus*, which may live in animals, have been found. They were isolated from clinical specimens of animal organs (Anwar *et al.*, 2015; Lim *et al.*, 2015; Onyiche *et al.*, 2018) or in the wild (Akagami *et al.*, 2007; Yoshiga, 2007). *Halicephalobus gingivalis* was a predominant parasite in horses, zebras, ruminants, and humans (Nadler *et al.*, 2003; Bhavesh Papadi *et al.*, 2013; Enemark *et al.*, 2016), and a synonym of *H. gingivalis* named *H. deletrix* was limited to horses and humans (Darien *et al.*, 1988; Rames *et al.*, 1995; Isaza *et al.*, 2000; Ondrejka *et al.*, 2010); both were found in more than 16 countries around the world (Onyiche *et al.*, 2018). While less reported, four species (*H. minutum*, *H. parvum*, *H. palmaris*, and *H. intermedia*) were isolated from plants in three countries (Germany, Brazil, and Russia). Four other species (*H. limuli* Timm, *H. similigaster*, *H. laticauda*, and *H. mephisto*) were sampled from water in five countries (Pakistan, Germany, Brazil, Russia, and Belgium) (Anderson *et al.,* 1998; Fronderie, 2023), and finally, *H. brevicauda* was from the soil in Uzbekistan (Akagami *et al.,* 2007; Fronderie, 2023). Thus, the distribution of *H. gingivalis* is broader than that of the other species, possibly because it lives in domestic animals that have been globally transferred everywhere. Within a host, the infected *H. gingivalis* individuals invaded organs through hematogenous spread (Brojer *et al.,* 2000; Papadi *et al.,* 2013; Henneke *et al.,* 2014) or semen and urine sediment (Kinde *et al.,* 2000). The infection of *H. gingivalis* could cause granulomas to fatal meningoencephalomyelitis. Clinical cures by anthelmintic treatments for horses infected by *H. gingivalis* were successful in a few cases (Dunn *et al.,* 1993; Pearce *et al.,* 2001; Muller *et al.,* 2008) but remained failures in many others (Anderson *et al.,* 1998; Fonderie *et al.,* 2012; Enemark *et al.,* 2016). Better protection from the infection of *Halicephalobus* species has potential through the understanding of their life history strategies.

In Vietnam, the distribution, infection, lifestyle, and cultivation of *Halicephalobus* species are unknown. As a previous result of the coincidence of *H. gingivalis* with horses, donkeys, ruminants, and humans (Onyiche *et al.,* 2018), we assumed that *H. gingivalis* and other *Halicephalobus* sp. could present in the habitats of those Bovidae mammals and they should live in the organismal composts (wastes and dead organs), which mixed with forest vegetation in the wild (here both are so-called vegetation samples) are a vector for the transmission of *Halicephalobus* sp. between different mammals. To address our curiosity, we conducted isolation and agar cultivation of *Halicephalobus* sp. wild-type strains of either *H. gingivalis* or *Halicephalobus* sp. in vegetation samples collected from two national parks, Cat Tien in the south and Cuc Phuong in the north of Vietnam. We used 18S rDNA sequences as regular barcodes for nematodes to analyze the potential divergence of the wild-type *Halicephalobus* isolates to reveal the variation among them.

MATERIALS AND METHODS

Isolation and cultivation of *Halicephalobus* nematodes

Isolation of nematodes was done as in our previous report (Le *et al.,* 2021). In brief, approximately 100 g of vegetation compost was collected from each site within two national parks, Cat Tien or Cuc Phuong in Vietnam. Each vegetation sample was kept in a zipper plastic bag at cool (20^{o}C to 25^{o}C) during transportation. In the laboratory, approximately 10 g to 20 g of each sample was placed on an agar plate of NGM seeded with *Escherichia coli* OP50 (called sample plate) and incubated for three days at room temperature (25^{o}C).

Generally, two gravid nematodes (isofemale), which were similar to *Caenorhabditis* species in size and presented on sample plates, were individually picked onto single media plates to grow strains (either NcM18, NcM12, or NGM + *E. coli* OP50 (Le *et al.,* 2021)). If none of the *Caenorhabditis*-like nematodes were observed for a few seconds, smaller larvae or individuals were chosen instead. The strains were given a numerical category.

Molecular identification

DNA extracts were prepared under a modified method of the single worm lysis protocol for *Caenorhabditis* nematodes (Ahringer, 2006). In brief, one to a few worms of each strain were lysed by putting in a PCR tube which had 20 µl of worm lysis buffer adding 1 µl of proteinase K. The worm solution was frozen in liquid nitrogen (-196^{o}C) for 10 min. Next, it was incubated at 62^{o}C for 90 min. The worm lysis was used for PCR to amplify the 18S rDNA sequence using the SSU18A (5′-AAAGATTAAGCCATGCATG-3′) and SSU26R primers (5′-CATTCTTGGCAAATGCTTTCG-3′) (Barriere, Felix, 2006). The PCR product was Sanger-sequenced (ATGC Company Ltd, Vietnam or Apical Scientific Sequencing Co, Malaysia). Following, the achieved sequences were aligned to the nucleotide database by nucleotide BLAST on the National Center for Biotechnology Information (NCBI) website (Zhang *et al.*, 2000).

Phylogenetic analysis

The phylogeny was formulated with the 18S rDNA sequences of *Halicephalobus* isolates. The phylogenetic tree was reconstructed by MEGA 11 software (Molecular Evolutionary Genetics Analysis) using the Neighbor-Joining method with 100 bootstrap replications (Tamura *et al.*, 2021).

RESULTS

Isolation of *Halicephalobus* sp.

We picked approximately 200 nematodes (mostly isofemales and several larvae), each to a separate agar media plate. Many of them grew well to be single strains with multiple generations, while many others did not develop and died on the bacteria agar media plates. To classify the taxon for each nematode strain, molecular methods were used for the identification of 18S rDNA sequences. Among the survivors, 66 strains were *Halicephalobus* sp., which were isolated from samples localized in 45 different sites in two national parks, and in particular, 40 strains from 26 out of 52 sites in Cuc Phuong National Park and 26 strains from 19 out of 40 sites in Cat Tien National Park (Fig. 1 and Table 1).

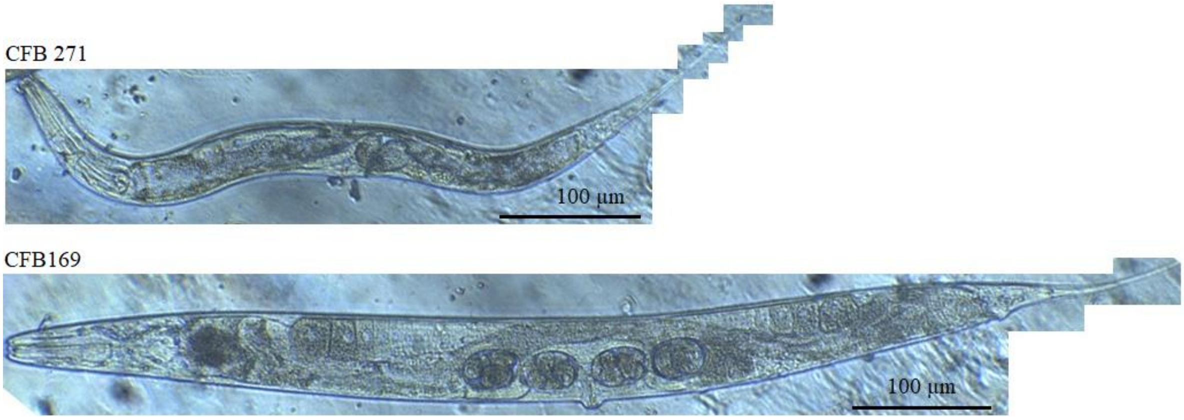

Figure 1. Examples of *Halicephalobus* species isolates (CFB271 and CFB169 adults).

All 66 *Halicephalobus* sp. strains in this research developed on either NGM or NcM18 media, providing a way to gain wild-type *Halicephalobus* sp. in the wild and to rear them in vitro on *E. coli* OP50 (Cooper 2017). They were found in vegetation samples whereas in many previous studies, *H. gingivalis* was reported to live in horses, zebras, donkeys, ruminants, and humans (Darien *et al.,* 1988; Rames *et al.,* 1995; Nadler *et al.,* 2003; Ondrejka *et al.,* 2010; Bhavesh Papadi *et al.,* 2013; Enemark *et al.,* 2016; Onyiche *et al.,* 2018; Noiva *et al.,* 2019; Avila *et al.,* 2020). Thus, they may live in the animals that inhabit in Cat Tien National Park and Cuc Phuong National Park rather than horses, zebras, and donkeys that never develop in the two parks (Murphy, Phan, 2002; Polet, Ling, 2004; Rugendyke, Nguyen, 2005).

Molecular determination

To describe the diversity of wild-type *Halicephalobus* sp. strains, we used part of the 18S rDNA sequences amplified with two specific primers (Table S1). The identity comparisons of 18S rDNA sequences for all 66 strains with the nucleotide database on NCBI showed they were either *H. gingivalis* or unknown *Halicephalobus* species for the first hit of the alignment result list (Table 1).

The 18S rDNA sequences were plotted together and they show a likely distorted distribution pattern (Fig. 2). Forty *Halicephalobus* strains from Cuc Phuong National Park (60.6%) are mostly biased toward one side of the phylogenic tree. Of them, twenty-eight strains (70%) are identical to the referred *Halicephalobus* sp. MK087054.1 [least 91.27% (CFB156) and last 97.63% (CFB89)], and five strains (12.5%) are identical to MK087055.1 [82.85% (CFB74) and 93.22% (CFB123)]. Of the seven left strains, three (7.5%) are identical to MK087053.1 [93.74% (CFB138) and 95.23% (CFB152)], three strains (7.5%) present similarity to MK087052.1 [79.02% (CFB125) and 89.48% (CFB128)], and finally only CFB86 (0.25%) is comparable with *H. gingivalis* JF706244.1 [88.57%] (Table 1).

Meanwhile, the majority of 26 strains from Cat Tien National Park (39.4%) were quite opposite in the tree. Ten strains (38.46%) have identity to *Halicephalobus* sp. MK087054.1 [89.46% (CFB184) and 99.66% (CFB228)] and 11 strains (42.3%) have that to MK087055.1 [79.33% (CFB183) and 89.64% (CFB179)]. Of the rest five strains, four (15.4%) are identical to MK08052.1 [87.99% (CFB187) and 91.85% (CFB166)] and last, CFB229 (0.38%) has similarity to MK087053.1 [94.88%] (Table 1).

Comparisons of 18S rDNA presented a divergence between the *Halicephalobus* isolates in the two parks in Vietnam and *H.* sp. isolates in other countries (in vast, MK087054.1 and MK087055.1). Thus, possibly *Halicephalobus* species ecologically adapt to the local hosts.

Halicephalobus species have different types of life histories. In previous reports, they completed the lifecycle in animals' organs (Hermosilla *et al*., 2011; Taulescu *et al*., 2016). Other *Halicephalobus* species might live in the wild and infect the host animals through open lesions (Cooper, Dholakia, 2017; Cooper *et al*., 2017). In this research, the rearing of *Halicephalobus* sp. strains on NcMs and NGM strongly suggests that they live and eat bacteria through life in environments. Our hypothesis is supported by previous findings of *Halicephalobus* species in soil (Anderson *et al.,* 1998; Akagami *et al.,* 2007; Fronderie, 2023).

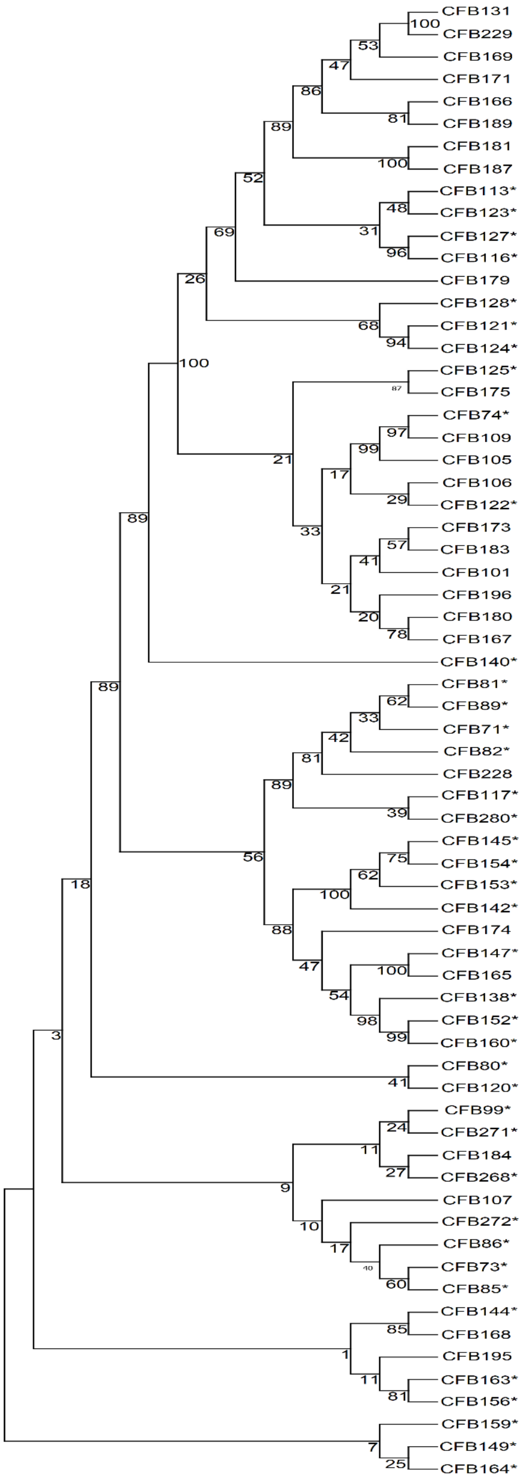

Figure 2. Phylogenetic tree of wild-type *Halicephalobus* sp. in two national parks. Phylogenesis was formulated for the unassembled sequences of 18S rDNA by the Neighbor-joining method. The numbers at joints are bootstrap values in 100 replicates. Asterisks-strains from Cuc Phuong National Park. Non-asterisk-strains from Cat Tien National Park.

Table 1. Site sampling and identity of each *Halicephalobus* sp. Strains.

No.	Site name	Global positioning system (GPS)	CFB No.	% identical to *Halicephalobus* sp.[†]	NCBI Genbank access
Cuc Phuong National Park					
1	CP2	20°20’56.7” N 105°35’48.1” E	CFB156	91.27%, MK087054.1	OQ625526
2			CFB153	96.41%, MK087054.1	OQ594694
3	CP4	20°20’56.7” N 105°35’46.5” E	CFB271	97.28%, MK087054.1	OQ594713
4	CP5	20°20’57.3” N 105°35’45.2” E	CFB127	94.28%, MK087054.1	OQ594685
5	CP7	20°20’58.4” N 105°35’36.5” E	CFB268	94.05%, MK087054.1	OQ594712
6			CFB280	96.68%, MK087054.1	OQ594715
7	CP12	20°17’40.0” N 105°39’57.5” E	CFB147	95.26%, MK087054.1	OQ594691
8			CFB120	90.24%, MK087054.1	OQ594679
9	CP14	20°17’41.5” N 105°39’58.4” E	CFB152	95.23%, MK087053.1	OQ594693
10			CFB145	96.22%, MK087054.1	OQ594690
11			CFB128	89.48%, MK087052.1	OQ594686
12			CFB121	88.54%, MK087052.1	OQ594680
13			CFB140	96.18%, MK087054.1	OQ683787
14			CFB142	96.18%, MK087054.1	OQ625525
15	CP17	20°15’8.40” N 105°42’32.0” E	CFB159	96.02%, MK087054.1	OQ594696
16	CP18	20°15’8.50” N 105°42’28.5” E	CFB154	95.93%, MK087054.1	OQ594695
17	CP20	20°15’51.2” N 105°41’57.6” E	CFB89	97.63%, MK087054.1	OQ594670
18	CP21	20°15’30.5” N 105°42’24.6” E	CFB82	97.06%, MK087054.1	OQ594667
19	CP22	20°15’24.9” N 105°42’28.7” E	CFB272	90.13%, MK087054.1	OQ594714
20	CP23	20°15’29.9” N 105°42’28.7” E	CFB124	85.28%, MK087055.1	OQ594683
21			CFB99	92.05%, MK087054.1	OQ594671
22	CP24	20°15’29.7” N 105°42’28.4” E	CFB81	97.31%, MK087054.1	OQ594666
23	CP25	20°15’28.4” N 105°42’35.2” E	CFB125	79.02%, MK087052.1	OQ594684
24	CP26	20°15’26.2” N 105°42’35.2” E	CFB122	84.77%, MK087055.1	OQ594681
25	CP27	20°15’24.8” N 105°42’35.0” E	CFB80	94.79%, MK087054.1	OQ594665

26	CP28	20°15’23.2” N 105°42’31.6” E	CFB149	95.28%, MK087054.1	OQ594692
27	CP30	20°15’7.60” N 105°42’45.0” E	CFB85	94.98%, MK087054.1	OQ594668
28	CP32	20°15’7.60” N 105°42’45.0” E	CFB116	95.06%, MK087054.1	OQ625527
29			CFB71	97.30%, MK087054.1	OQ594662
30	CP33	20°14’53.6” N 105°42’35.9” E	CFB160	95.02%, MK087053.1	OQ625520
31	CP34	20°14’53.5” N 105°42’34.3” E	CFB144	92.09%, MK087054.1	OQ594689
32	CP35	20°14’53.9” N 105°42’37.0” E	CFB123	93.22%, MK087055.1	OQ594682
33			CFB113	84.56%, MK087055.1	OQ594677
34			CFB73	93.13%, MK087054.1	OQ594663
35	CP36	20°14’59.8” N 105°42’28.1” E	CFB86	88.57%, *H. gingivalis* JF706244.1	OQ594669
36	CP37	20°14’56.7” N 105°42’21.1” E	CFB74	82.85%, MK087055.1	OQ594664
37	CP39	20°14’40.4” N 105°42’25.2” E	CFB138	93.74%, MK087053.1	OQ594688
38			CFB117	97.54%, MK087054.1	OQ594678
39	CP50	Missing determination	CFB163	92.15%, MK087054.1	OQ625522
40			CFB164	92.23%, MK087054.1	OQ625523
Cat Tien National Park					
41	CT3	11°23’18.8” N 107°21’3.6” E	CFB184	89.46%, MK087054.1	OQ594706
42			CFB196	79.95%, MK087055.1	OQ594709
43	CT5	11°23’26.0” N 107°21’1.80” E	CFB109	83.82%, MK087055.1	OQ594676
44	CT9	11°23’39.7” N 107°21’47.9” E	CFB171	93.79%, MK087054.1	OQ650164
45	CT14	11°24’21.4” N 107°24’25.6” E	CFB166	91.85%, MK087052.1	OQ594698
46	CT15	11°24’20.9” N 107°24’22.3” E	CFB106	81.47%, MK087055.1	OQ594674
47	CT19	11°28’46.4” N 107°22’52.9” E	CFB105	84.23%, MK087055.1	OQ594673
48	CT22	11°27’56.1” N 107°22’43.5” E	CFB180	80.70%, MK087055.1	OQ594703
49	CT28	11°26’56.6” N 107°21’35.9” E	CFB183	79.33%, MK087055.1	OQ594705
50	CT29	11°27’15.2” N 107°21’18.3” E	CFB131	94.04%, MK087054.1	OQ594687
51	CT30	11°27’32.8” N 107°20’44.2” E	CFB195	91.62%, MK087054.1	OQ625521

52	CT32	11°27’5.00” N 107°21’25.6” E	CFB228	99.66%, MK087054.1	OQ594710
53	CT34	11°27’17.9” N 107°22’5.60” E	CFB229	94.88%, MK087053.1	OQ594711
54	CT37	11°27’33.8” N 107°26’52.9” E	CFB165	94.61%, MK087054.1	OQ594697
55	CT39	11°27’51.8” N 107°27’42.1” E	CFB187	87.99%, MK087052.1	OQ594707
56			CFB175	79.83%, MK087055.1	OQ594701
57			CFB181	88.72%, MK087052.1	OQ594704
58			CFB189	90.92%, MK087052.1	OQ594708
59			CFB169	95.37%, MK087054.1	OQ594699
60	CT42	11°26’13.9” N 107°25’25.0” E	CFB167	81.38%, MK087055.1	OQ650163
61	CT45	11°25’41.2” N 107°25’37.2” E	CFB101	81.51%, MK087055.1	OQ594672
62	CT49	11°25’50.7” N 107°25’45.9” E	CFB174	93.71%, MK087054.1	OQ625518
63			CFB168	92.38%, MK087054.1	OQ625519
64	CT51	11°24’48.2” N 107°25’25.9” E	CFB179	89.67%, MK087055.1	OQ594702
65	CT52	11°25’26.4” N 107°25’45.1” E	CFB107	96.05%, MK087054.1	OQ594675
66			CFB173	80.02%, MK087055.1	OQ594700

† The nucleotide sequence alignment showed a percentage of the 1st hit with the *Halicephalobus* species which was not isolated in this research. The BLASTnt was conducted on the NCBI website on March 24, 2023.

CONCLUSION

We isolated and molecularly determined 66 wild-type parasitic nematode strains of *Halicephalobus* sp. freely living in vegetation composts from two national parks. They are segregated, indicating that the distinct local host animals might play a key role in the nematode divergence in the ecological systems (forests). These parasites could invade animals rather than only horses, ruminants, and humans, causing granulomas to fatality. Thus, it is prudent for humans to visit forests and consume raw forestry products.

Acknowledgment: *We thank the staffs at Cuc Phuong and Cat Tien National Parks for sample guides and collections.*

REFERENCE

Ahringer J (2006) Reverse genetics. *WormBook*. WormBook, The *C. elegans* Research Community.

Akagami M, Shibahara T, Yoshiga T, Tanaka N, Yaguchi Y, Onuki T, Kondo T, Yamanaka T, Kubo M (2007) Granulomatous nephritis and meningoencephalomyelitis caused by *Halicephalobus gingivalis* in a pony gelding. *J Vet Med Sci* 69(11): 1187-1190.

Anderson RC, Linder KE, Peregrine AS (1998) *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. *Parasite* 5(3): 255-261.

Anwar MA, Gokozan HN, Ball MK, Otero J, McGwire BS (2015) Fatal human eosinophilic meningo-encephalitis caused by CNS co-infection with *Halicephalobus gingivalis* and West Nile virus. *Parasitol Int* 64(5): 417-420.

Avila VA, Lopez-Garcia Y, Hernandez-Castro R, Salas-Garrido CG, Ramirez-Lezama J, Calderon-Villa R, Martinez-Chavarria LC (2020) First case of fatal equine meningoencephalitis caused by Halicephalobus gingivalis in Mexico. *Vet Parasitol Reg Stud Reports* 20: 100399.

Barriere A, Felix MA (2006) Isolation of C. elegans and related nematodes. *WormBook*. T. C. e. R. Community, WormBook**:** 1-9.

Bhavesh Papadi, Carole Boudreaux, J. Allan Tucker, Blaine Mathison, Henry Bishop, Eberhard ME (2013) Case report: *Halicephalobus gingivalis*: A rare cause of Meningoencephalomyelitis in Humans. *Am. J. Trop Med Hyg* 88(6): 2.

Brojer JT, Parsons DA, Linder KE, Peregrine AS, Dobson H (2000) *Halicephalobus gingivalis* encephalomyelitis in a horse. *Can Vet J* 41(7): 559-561.

Cooper AJR, Dholakia S (2017) Helminths in organ transplantation - Authors' reply. *Lancet Infect Dis* 17(6): 583.

Cooper AJR, Dholakia S, Holland CV, Friend PJ (2017) Helminths in organ transplantation. *Lancet Infect Dis* 17(6): e166-e176.

Darien BJ, Belknap J, Nietfeld J (1988) Cerebrospinal fluid changes in two horses with central nervous system nematodiasis (*Micronema deletrix*). *J Vet Intern Med* 2(4): 201-205.

Dunn DG, Gardiner CH, Dralle KR, Thilsted JP (1993) Nodular granulomatous posthitis caused by *Halicephalobus* (syn. *Micronema*) sp. in a horse. *Vet Pathol* 30(2): 207-208.

Enemark HL, Hansen MS, Jensen TK, Larsen G, Al-Sabi MN (2016) An outbreak of bovine meningoencephalomyelitis with identification of *Halicephalobus gingivalis*. *Vet Parasitol* 218: 82-86.

Fonderie P, Bert W, Hendrickx F, Houthoofd W, Moens T (2012) Anthelmintic tolerance in free-living and facultative parasitic isolates of *Halicephalobus* (Panagrolaimidae). *Parasitology* 139(10): 1301-1308.

Fronderie P (2023). Biology and evolutionary history of the facultative parasitic namtode *Halicephalobus gingivalis*, Ghent University.

Henneke C, Jespersen A, Jacobsen S, Nielsen MK, McEvoy F, Jensen HE (2014) The distribution pattern of *Halicephalobus gingivalis* in a horse is suggestive of a haematogenous spread of the nematode. *Acta Vet Scand* 56(1): 56.

Hermosilla C, Coumbe KM, Habershon-Butcher J, Schoniger S (2011) Fatal equine meningoencephalitis in the United Kingdom caused by the panagrolaimid nematode *Halicephalobus gingivalis*: case report and review of the literature. *Equine Vet J* 43(6): 759-763.

Isaza R, Schiller CA, Stover J, Smith PJ, Greiner EC (2000) *Halicephalobus gingivalis* (Nematoda) infection in a Grevy's zebra (*Equus grevyi*). *J Zoo Wildl Med* 31(1): 77-81.

Kinde H, Mathews M, Ash L, St Leger J (2000) *Halicephalobus gingivalis* (*H. deletrix*) infection in two horses in southern California. *J Vet Diagn Invest* 12(2): 162-165.

Le T S, Nguyen TTH, Thi Mai Huong B, Nguyen HG, Ha BH, Nguyen VS, Nguyen MH, Nguyen HH, Wang J (2021) Cultivation of *Caenorhabditis elegans* on new cheap monoxenic media without peptone. *J Nematol* 53.

Lim CK, Crawford A, Moore CV, Gasser RB, Nelson R, Koehler AV, Bradbury RS, Speare R, Dhatrak D, Weldhagen GF (2015) First human case of fatal *Halicephalobus gingivalis* meningoencephalitis in Australia. *J Clin Microbiol* 53(5): 1768-1774.

Muller S, Grzybowski M, Sager H, Bornand V, Brehm W (2008) A nodular granulomatous posthitis caused by *Halicephalobus* sp. in a horse. *Vet Dermatol* 19(1): 44-48.

Murphy D, Phan DT (2002). *Mammal observations in Cat Tien National Park, Vietnam*. The Cat Tien National Park Conservation Project, The ministry of Agriculture and Rural Development (Hanoi) and the WWF-Indochina Programme.

Nadler SA, Carreno RA, Adams BJ, Kinde H, Baldwin JG, Mundo-Ocampo M (2003) Molecular phylogenetics and diagnosis of soil and clinical isolates of *Halicephalobus gingivalis* (Nematoda: Cephalobina: Panagrolaimoidea), an opportunistic pathogen of horses. *Int J Parasitol* 33(10): 1115-1125.

Noiva R, Ruivo P, de Carvalho LM, Fonseca C, Fevereiro M, Carvalho P, Orge L, Monteiro M, Peleteiro MC (2019) First description of a fatal equine infection with *Halicephalobus gingivalis* in Portugal. Relevance for public health. *Vet Med Sci* 5(2): 222-229.

Ondrejka SL, Procop GW, Lai KK, Prayson RA (2010) Fatal parasitic meningoencephalomyelitis caused by *Halicephalobus deletrix* : a case report and review of the literature. *Arch Pathol Lab Med* 134(4): 625-629.

Onyiche TE, Okute TO, Oseni OS, Okoro DO, Biu AA, Mbaya AW (2018) Parasitic and zoonotic meningoencephalitis in humans and equids: Current knowledge and the role of *Halicephalobus gingivalis*. *Parasite Epidemiol Control* 3(1): 36-42.

Papadi B, Boudreaux C, Tucker JA, Mathison B, Bishop H, Eberhard ME (2013) H*alicephalobus gingivalis*: a rare cause of fatal meningoencephalomyelitis in humans. *Am J Trop Med Hyg* 88(6): 1062-1064.

Pearce SG, Boure LP, Taylor JA, Peregrine AS (2001) Treatment of a granuloma caused by *Halicephalobus gingivalis* in a horse. *J Am Vet Med Assoc* 219(12): 1735-1738, 1708.

Polet G, Ling S (2004) Protecting mammal diversity: opportunities and constraints for pragmatic conservation management in Cat Tien National Park, Vietnam. *Oryx* 38(No 2).

Rames DS, Miller DK, Barthel R, Craig TM, Dziezyc J, Helman RG, Mealey R (1995) Ocular *Halicephalobus* (syn. *Micronema*) deletrix in a horse. *Vet Pathol* 32(5): 540-542.

Rugendyke B, Nguyen TS (2005) Conservation costs: Nature-based tourism as development at Cuc Phuong National Park, Vietnam. *Asia Pacific Viewpoint* 46(No. 2): 5.

Tamura K, Stecher G, Kumar S (2021) MEGA11: Molecular Evolutionary Genetics Analysis Version 11. *Mol Biol Evol* 38(7): 3022-3027.

Taulescu MA, Ionica AM, Diugan E, Pavaloiu A, Cora R, Amorim I, Catoi C, Roccabianca P (2016) First report of fatal systemic *Halicephalobus gingivalis* infection in two Lipizzaner horses from Romania: clinical, pathological, and molecular characterization. *Parasitol Res* 115(3): 1097-1103.

Yoshiga Y (2007) Detection of *Halicephalobus gingivalis* in soil nematode samples using PCR. *Japanese J Nematol* 37(2): 3.

Zhang Z, Schwartz S, Wagner L, Miller W (2000) A greedy algorithm for aligning DNA sequences. *J Comput Biol* 7(1-2): 203-214.