

Nematicidal activity of Equine milk in *Caenorhabditis elegans*



Correia DS^{1*}, Argumedo AK¹, Queiroz CL¹, Morais LPR², Moreira MAS¹ and Araujo JV¹

¹Department of Veterinary, Federal University of Viçosa, Viçosa, Brazil

²Department of Administration and Accounting, Federal University of Viçosa, Viçosa, Brazil

Submission: December 20, 2024, Published: January 10, 2025

*Corresponding author: Douglas C Souza, Department of Veterinary, Federal University of Viçosa, Viçosa, Minas Gerais, Brazil

Abstract

The present study investigated the nematicidal activity of equine milk using the model nematode *Caenorhabditis elegans* (*C. elegans*). Samples of mare's, goat's and cow's milk were sampled in parallel, in addition to UHT bovine milk used as a negative control. Equine milk samples demonstrated significant nematicidal activity, with an average nematode survival rate of 31% after 24 hours of exposure. In comparison, goat's milk showed an average of 64% survival, while bovine milk did not exhibit relevant nematicidal activity, with a survival rate of 94%, consistent with the negative control. This study brings to the literature, for the first time, the nematicidal potential of equine milk, highlighting its value as a natural agent for parasite control, aligning with sustainable practices and the principles of the One Health concept.

Keywords: Animal health; biological control; nematodes; One Health

Introduction

The use of natural agents as sustainable alternatives for controlling microorganisms that negatively impact human and animal health and agricultural management has received increasing attention as an innovative and promising strategy. This interest arises in response to the growing antimicrobial resistance and the harm to human, animal, and environmental health caused by the excessive use of pesticides [1,2].

In this context, milk and its derivatives have stood out as a highly relevant resource due to their bioactive properties of interest in health. These properties include antimicrobial and anti-inflammatory activities, widely evidenced in different species [3,4]. Notable examples, such as goat and camel milk, have demonstrated high efficacy in generating mortality in different nematodes, highlighting their potential for sustainable parasite control as well as the development of natural therapeutic alternatives with additional health benefits [5,6].

Equine milk, as well as that from other animals, is already known for its nutritional and therapeutic properties [7]. Rich in nutrients and chemically similar to human milk, it is an alternative to cow's milk, especially for people with IgE-mediated allergies. In

Italy, for individuals with allergies, equine milk is recommended as a substitute for cow's milk [8]. Additionally, equine milk has significant bioactivities and is traditionally used in Central Asia to treat digestive and cardiovascular diseases.

Recent studies have demonstrated the model nematode *C. elegans* as an indicator of nematicidal bioactivity in various natural materials, including milk. This approach is accessible and efficient for initial experimental screenings [9-11]. Thus, the present study evaluated the nematicidal activity of milk from different species, using *C. elegans* as a model. The primary focus was equine milk, whose nematicidal activity had not yet been described in the literature.

Material and Methods

Milk samples

This study was carried out in accordance with the ethical guidelines for the use of animals and was approved by the Animal Ethics Committee (CEUA) of the Federal University of Viçosa under protocol number 55/2019. Milk from mares (n=7), cows (n=10), and goats (n=10) was used in exposure tests to the model nematode. Milk from mares and cows was collected at the

Veterinary Hospital of the Department of Veterinary Medicine of UFV and from farms in the surrounding region, while goat milk samples were obtained from the goat sector of the Department of Animal Science of the Federal University of Viçosa. This sector is also located in Viçosa, Minas Gerais, Brazil. UHT (Ultra-High-Temperature Processing) cow milk from different batches (n=10) purchased from local stores was used as a negative control due to its similarity to milk, but without high nematocidal activity, as previously evidenced [10]. Samples were kept at 4°C until the time of analysis, with sample collection and exposure procedures initiated within 4 hours of milking. [12,13]

Nematode culture and synchronization

C. elegans Bristol N2 and *Escherichia coli* (*E. coli*) OP50 from CGC (Caenorhabditis Genetics Center) were kindly donated by Dr. Francine Côa (USP). The culture media and manipulation of the organisms were performed using conditional techniques. The worms were maintained in a B.O.D incubator at 20°C on nematode growth medium (NGM) plates (1.7% Bacto agar, 0.5% Bacto peptone, 50 mM NaCl, 25 mM potassium phosphate-free pH 6.0, 1 mM CaCl₂, 1 mM MgSO₄ and 5 µg/mL cholesterol, H₂O for 1 liter). The (*E. coli*) OP50 strain was used as a food source [14].

To synchronize age- and stage-matched *C. elegans* strains, gravid adult worms were exposed to sterile water containing 0.5 M NaOH and 4% bleach solution (NaOH), this solution was prepared at the time of use. Eggs were released and isolated by vortexing, then centrifuged (1,300 × g for 1 min) and washed 3 times in M9 releases (3 g KH₂PO₄, 6 g Na₂HPO₄, 5g NaCl, 1 ml 1 M MgSO₄, H₂O to 1 liter). After the last wash, eggs were suspended in S Basal (5.85 g NaCl, 1 g K₂HPO₄, 6 g KH₂PO₄, 1 ml cholesterol (5 mg/ml in ethanol), H₂O to 1 liter) under gentle absorption at 20°C for 4 h. This process aims at egg hatching. With lack of food, the animals do not develop, remaining as L1 larvae. After the eggs have fully hatched, the larvae are cultured on NGM agar with (*E. coli*) OP50 at 20°C for ~60 h until they reach the L4 stage [15]. After this period, the organisms were collected and washed 3 times in S.

Basal, centrifuged at (1300×g for 2 min) and the suspension was adjusted so that each 50µl contained ~400 L4 worms.

Experimental design

The nematodes were transferred in triplicate for each reading, with 50µl of nematode suspension added to each well of a 96-well plate containing 50µl of the milk samples diluted in M9 buffer. The plates were then incubated at 20°C. For each reading, at least 100 nematodes were recovered and analyzed using direct microscopy. To determine the survival rate of *C. elegans*, the number of live worms was recorded after 24 hours, and the percentage of surviving worms was calculated using the following formula: Survival (%) = (live worms / total worms used) × 100. Worms were considered dead if they did not respond to stimulation with a platinum wire pick.

Statistics Analysis

Microsoft Excel software was used for statistical analyses. Comparisons between the controls and exposed groups were conducted using one-way analysis of variance (ANOVA). Differences were considered significant when $p < 0.05$ [16,17].

Results

The survival of *C. elegans* after 24 hours of exposure to different types of milk was assessed, including equine, bovine, goat, and UHT bovine milk, which was used as the negative control. The results showed significant variation in the survival rates across the milk samples analyzed. Equine milk exhibited the lowest survival rate, with an average of 31% nematode survival. Bovine milk, as previously shown, did not display nematocidal activity, with a survival rate of 94%, and no statistically significant differences were observed between the UHT bovine milk samples tested ($p \leq 0.05$). Goat milk showed an average survival rate of 64%. A statistically significant difference in mortality was observed between the equine and goat milk samples when compared to the negative control ($p \leq 0.05$). (Table 1)

Table 1: Survival results of *Caenorhabditis elegans* after 24 hours of exposure to equine, goat, cow, and UHT bovine milk samples (negative control).

Sample	% Survival					
	Replicate 1	Replicate 2	Replicate 3	Mean	Standard Deviation	Standard Error
Equine Milk (n=7)	31%	32%	31%	31%**	58%	33%
Bovine Milk (n=10)	93%	94%	94%	94%*	58%	33%
Goat Milk (n=10)	63%	66%	64%	64%**	153%	88%
UHT Milk (Negative Control) (n=10)	97%	98%	99%	98%	100%	58%

Values marked with (*) show no statistical difference compared to the negative control. Values marked with (**) show a statistical difference compared to the negative control. Both values marked with (*) and (**) indicate a statistically significant difference ($p < 0.05$; One-way ANOVA, Tukey post-test).

Discussion

As a nematode, *C. elegans* shares biological characteristics with parasitic helminths, making it a valuable tool for preclinical research on nematocidal agents. This is particularly relevant due to the difficulties associated with cultivating parasitic nematodes, especially because of the need for a secondary host organism [16].

The consumption of equine milk, unconventional in the West, is gaining attention as a food source and antipathogenic agent, with the potential to complement or even replace traditional allopathic treatments. This is especially significant in the context of organic livestock management, which aligns with the concept of One Health by promoting natural, economically viable, and socially participatory practices [1].

The composition of milk varies between species, influenced by genetic, physiological, and environmental factors. In a previous study, Saanen goats exhibited higher nematocidal activity in the milk produced compared to other breeds [10]. However, the activity varied between individuals, with some animals showing samples similar to negative controls. Therefore, evaluating the nematocidal activity of each animal is essential for efficient use of milk. Among the milk samples tested in the present study, equine milk also emerged as the primary focus of nematocidal activity. Although equine milk has not been widely studied, our results demonstrate that it possesses significant nematocidal activity, with *C. elegans* showing a notably low survival rate when exposed to it.

Given the variation in activity observed between different species and individuals, it is essential to investigate in depth the molecular mechanisms behind this activity and its potential for applications in various fields, including natural pathogen control and therapeutic uses. Tests on parasitic nematodes, followed by evaluating the ability to reduce infections caused by these pathogens in mammals, are needed to fully understand the potential of equine milk as a therapeutic agent and assess its efficacy in controlling parasitic infections in animals and humans [17]. These findings suggest that equine milk has natural nematocidal potential, making it a promising biotechnological agent. This contribution is particularly relevant given the growing interest in equine milk, both for its unique nutritional and therapeutic properties, including its potential applications in the food and pharmaceutical industries.

Conclusion

For the first time, this study was able to assess the nematocidal activity of equine milk using *C. elegans*. Our results reveal that equine milk exhibits significant nematocidal effects, as evidenced by the notably low survival rate of *C. elegans* upon exposure. This finding has the potential to move forward with the knowledge and technologies that use the potential of equine milk in the field and industry.

Acknowledgment

This research was supported by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Fundação de Amparo à Pesquisa do Estado de Minas Gerais (Fapemig) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (Capes) Finance code 001.

References

- Collignon Pj, McEwen Sa (2019) One Health-Its Importance in Helping to Better Control Antimicrobial Resistance. *Tropical Medicine and Infectious Disease* 4(1): 22-29.
- Velazquez-Meza, Me (2022) Antimicrobial resistance: One Health approach. *Veterinary World* Pp: 743-749.
- Biadała A (2020) Antimicrobial activity of goat's milk fermented by single strain of kefir grain microflora. *European Food Research and Technology* 246(6):1231-1239.
- Azizkhani M, Saris PEJ, Baniasadi M (2021) An in-vitro assessment of antifungal and antibacterial activity of cow, camel, ewe, and goat milk kefir and probiotic yogurt. *Journal of Food Measurement and Characterization* 15(1): 406-415.
- Alimi D (2016) First report of the in vitro nematocidal effects of camel milk. *Veterinary Parasitology* 228: 153-159.
- Alimi D, Rekik M, Akkari H (2019) Comparative in vitro efficacy of kefir produced from camel, goat, ewe and cow milk on *Haemonchus contortus*. *Journal of Helminthology* 93(4): 440-446.
- Barreto IMLG (2019) Equine milk and its potential use in the human diet. *Food Science and Technology* 39(1): 1-7.
- Musaev A (2021) Mare's Milk: Composition, its Properties and Uses in Medicine. *Archives of Razi Institute*.
- Hahnel SR (2020) *Caenorhabditis elegans* in anthelmintic research – Old model, new perspectives. *International Journal for Parasitology: Drugs and Drug Resistance* 14: 237-248.
- Correia DS (2023) Nematocidal Activity of Goat Milk in *Caenorhabditis elegans*. *Journal of Dairy & Veterinary Sciences* 15(3): 1-5.
- Mudd N, Liceaga AM (2022) *Caenorhabditis elegans* as an in vivo model for food bioactives: A review. *Current Research in Food Science* 5: 845-856.
- Stiernagle T (2006) Maintenance of *C. elegans*. *Worm Book*.
- Gubert P (2022) Guia para pesquisadores iniciantes em *Caenorhabditis elegans*. [s.l.] Publicar Editora.
- Burns AR (2015) *Caenorhabditis elegans* is a useful model for anthelmintic discovery. *Nature Communications* 6(1): 7485.
- Alimi D (2018) In vivo nematocidal potential of camel milk on *Heligmosomoides polygyrus* gastro-intestinal nematode of rodents. *Helminthologia* 55(2):112-118.
- Fay DSA (2013) biologist's guide to statistical thinking and analysis. *Worm Book* p: 1-54.
- Sant'anna V, Vommaro RC, De Souza W (2013) *Caenorhabditis elegans* as a model for the screening of anthelmintic compounds: Ultrastructural study of the effects of albendazole. *Experimental Parasitology* 135(1):1-8.



This work is licensed under Creative Commons Attribution 4.0 License
DOI: [10.19080/JDVS.2025.17.555964](https://doi.org/10.19080/JDVS.2025.17.555964)

**Your next submission with Juniper Publishers
will reach you the below assets**

- Quality Editorial service
- Swift Peer Review
- Reprints availability
- E-prints Service
- Manuscript Podcast for convenient understanding
- Global attainment for your research
- Manuscript accessibility in different formats
(Pdf, E-pub, Full Text, Audio)
- Unceasing customer service

Track the below URL for one-step submission

<https://juniperpublishers.com/online-submission.php>