International Journal of Infectious Diseases 95 (2020) 216–220

Contents lists available at ScienceDirect

International Journal of Infectious Diseases

journal homepage: www.elsevier.com/locate/ijid

Cooking copepods: The survival of cyclopoid copepods (Crustacea: Copepoda) in simulated provisioned water containers and implications for the Guinea Worm Eradication Program in Chad, Africa

Christopher A. Cleveland[a,b,*,1], Kayla B. Garrett[a,**,1], Erin K. Box[a], Zavier Eure[a,c], Ania A. Majewska[d], Jessica A. Wilson[b], Michael J. Yabsley[a,b]

[a] Southeastern Cooperative Wildlife Disease Study, Veterinary Medicine, University of Georgia, 589 D.W. Brooks Dr., Athens, GA 30601, USA
[b] Warnell School of Forestry and Natural Resources, University of Georgia, 180 E. Green St., Athens, GA 30602, USA
[c] College of Agricultural and Environmental Sciences, North Carolina Agricultural and Technical State University, 1601 East Market St., Greensboro, NC 27411, USA
[d] Emory University, 201 Dowman Dr., Atlanta, GA 30322, USA

ARTICLE INFO

Article history:
Received 2 January 2020
Received in revised form 4 March 2020
Accepted 6 March 2020

Keywords:
Copepod survival
Domestic dogs
Dracunculus medinensis
Guinea worm
Water
Transmission

ABSTRACT

Introduction: The global Guinea Worm Eradication Program has reduced numbers of human infections of Guinea worm disease (dracunculiasis) to 49 cases in four countries. However, infections of domestic animals (dogs and cats) have recently been recognized and are increasing. Typically, Guinea worm (*Dracunculus medinensis*) transmission occurs via the ingestion of copepods from water. Despite several interventions, including tethering of dogs while worms emerge, the number of infected dogs continue to increase. One hypothesis is that dogs could be infected through the ingestion of copepods in provisioned water.
Objectives: The purpose of this study was to determine whether copepods can survive in water containers under typical Chadian temperatures.
Methods: Four container types (plastic, glass, gourd, and metal) were seeded with copepods and exposed to simulated Chadian temperatures.
Results: All copepods in the metal containers died within 4 h. Conversely, after 8 h live copepods were still present in plastic, glass, and gourd containers.
Conclusions: If provisioned water is provided to potential hosts of *D. medinensis*, metal containers create the most inhospitable environment for copepods. Plastic containers have little effect on copepod mortality. The use of metal containers for water provisions could be a useful tool assisting with the interruption of *D. medinensis* transmission among dogs.

Introduction

The Guinea Worm Eradication Program, currently led by The Carter Center (Atlanta, GA, USA), has been successful in reducing the number of human cases of Guinea worm disease (dracunculiasis, caused by the nematode *Dracunculus medinensis*) from 3.5 million in 21 countries in the 1980s to 49 cases in four countries (Chad, South Sudan, Cameroon, and Angola) thus far in 2019 (WHO Collaborating Center for Research Training and Eradication of Dracunculiasis, 2019). This program has experienced great success, especially in the face of political instability in some endemic countries (Awofeso, 2013), but now faces a new challenge: the infection of peri-domestic dogs and cats, primarily in Chad, Africa (Eberhard et al., 2014; Hopkins et al., 2017; Thiele et al., 2018). In 2019, there was a total of 1,927 dog and 46 cat infections in Chad, and several domestic animal cases in Ethiopia

* Corresponding author at: Southeastern Cooperative Wildlife Disease Study, Veterinary Medicine, University of Georgia, 589 D.W. Brooks Dr., Athens, GA 30601, USA.
** Corresponding author.
E-mail address: ccleve@uga.edu (C.A. Cleveland).
[1] Christopher A. Cleveland and Kayla B. Garrett contributed equally.

https://doi.org/10.1016/j.ijid.2020.03.016

and Mali (WHO Collaborating Center for Research Training and Eradication of Dracunculiasis, 2020).

The route of transmission to these animals remains unknown. Classical transmission of *D. medinensis* is by the ingestion of an obligate intermediate host (cyclopoid copepod) infected with third-stage *D. medinensis* larvae (L3) via drinking water (Cairncross et al., 2002). Recent work has shown that dogs may ingest copepods under experimental settings (Garrett et al., 2020); however, the occurrence of dog and cat infections in Chad does not fit the classical epidemiology observed in water-borne transmission events, i.e., a point source infection with closely clustered subsequent infections (Eberhard et al., 2014). Furthermore, several recent studies have indicated that certain aquatic animals may play a role in transmission by serving as paratenic or transport hosts (Eberhard et al., 2016a; Eberhard et al., 2016b; Cleveland et al., 2017; Cleveland et al., 2019). The ingestion of amphibians or fish harboring L3 could help explain why patterns of infections among dogs and cats are different from those historically observed in human cases.

Due to the increase in dog cases, new interventions aimed at interrupting transmission have been suggested and implemented in many villages where infections have been documented. One intervention is to tether dogs while a worm is emerging to prevent them from visiting water bodies that may contain copepods. Alternatively, proactively tethering dogs prior to the peak transmission season in Chad (March–June) has also been discussed as a potential intervention to decrease the risk of Guinea worm transmission. Provisioning of water for dogs during tethering is necessary given that ambient temperatures in Chad during the peak *D. medinensis* transmission period can reach 43 °C (Nematchoua et al., 2019). Ideally, provisioned water would be obtained from a safe water source (e.g., bore well). However, some individuals may not choose to use safe water sources or they may not be available in a particular village, in which case water from nearby natural water sources (e.g., ponds or small lagoons) containing copepods infected with *D. medinensis* may be used. Further, despite being provisioned with water filters to exclude copepods from water, not all villagers adhere to this intervention, nor does every single person collecting water in Chad have a copepod filter.

As dogs are capable of ingesting copepods when drinking from a container, especially if copepod concentrations are high (Garrett et al., 2020), the transmission of *D. medinensis* may occur if infected copepods are accidentally collected during water retrieval. Furthermore, provisioned water for livestock, such as goats and chickens, is another source of water consumption for dogs. Drinking from this unintended source has been noted in many villages reporting dog *D. medinensis* infections (Cleveland, personal communication). Due to the extreme temperatures in Chad, we believe that provisioned water containers may create variable conditions for copepods over the course of a day; however, copepod survival has not been evaluated under these conditions. The objective of this study was to determine whether copepods in provisioned water containers would survive when exposed to typical Chadian environmental conditions. Additionally, it was aimed to determine whether container material was related to copepod mortality. The identification of material(s) that maximize copepod mortality would provide information to the Guinea Worm Eradication Program on the best practices of water provisioning for dogs.

Materials and methods

Prior to developing the experimental system, eight ponds in Chad were visited during the peak transmission season of Guinea worm (March–June), and temperatures were taken 1 meter from the shore at the surface and 0.5 meters below the surface. The surface temperatures averaged 34.6 °C, and the sub-surface temperatures averaged 30.2 °C. Based on this information, we chose 34 °C as our system starting temperature. Previous work involving *Macrocyclops* species has shown an upper thermal threshold of 40 °C in a subtropical environment (Rey et al., 2004) and we therefore felt confident that the copepods used in our trial could approximate those conditions found in Chad (which can also be classified as subtropical).

Four experimental systems were used to mimic water containers provisioned to animals in Chad, Africa (Figure 1). A system consisted of a sand base (mimicking similar reflective and heating conditions that a container would be exposed to in a Chadian village), a UVA/UVB bulb to heat the system, one of four container types (2.5 liter glass container (Pyrex), 2.5 liter metal container, 2.5 liter white plastic container, 2.5 liter gourd container), a thermometer for the water, and a thermometer for the system temperature and percentage humidity. A regulating digital thermostat (Century Products Inc., El Monte CA, USA) was used to maintain the system at 38–40 °C and the system was allowed to heat for 15 min to reach this temperature prior to copepod exposure.

One liter of dechlorinated water per system was heated to 34 °C to mimic the typical daytime surface water temperature of ponds in Chad. Laboratory-reared copepods (*Macrocyclops* sp, $n = 100$) were added to each liter of warmed water to allow for temperature acclimation. Copepods and heated water were added to the container in the system and exposed to heat bulbs uninterrupted for 2, 4, 6, or 8 h . Water temperature, system temperature, system percentage humidity, ambient room temperature, and copepod activity were recorded hourly. All systems were located away from windows or air vents to reduce fluctuations in these variables.

At the end of each exposure period, the containers were removed from the heating systems, final measurements of water temperature, system temperature, and percentage humidity were recorded, and the dishes removed from the systems (Table 1).

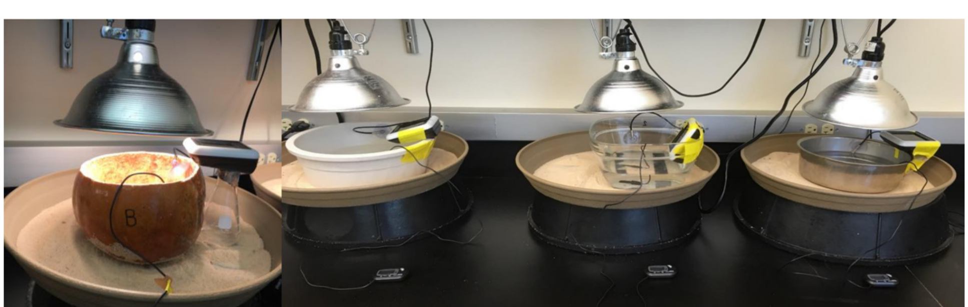

Figure 1. Experimental set-up to investigate the effect of temperature and dish type (metal, plastic, glass, and gourd) on copepod mortality.

Table 1
Range and average water temperatures of trial systems at the end of the copepod survival experiment replicates (n = 6) using *Macrocyclops* sp.

	Trial length (h)	Water temperature (°C)		
		Max.	Min.	Avg.
Glass	2	41.4	36.3	32.8
	4	40.5	37.2	38.4
	6	41.0	35.0	38.2
	8	40.9	34.6	39.0
Gourd	2	37.4	23.3	33.7
	4	45.1	36.6	39.8
	6	39.2	36.2	37.5
	8	48.6	34.1	41.2
Metal	2	41.7	38.1	39.7
	4	42.0	39.3	40.3
	6	43.1	39.1	40.9
	8	42.6	38.4	40.7
Plastic	2	38.7	36.5	37.4
	4	40.1	36.4	38.0
	6	38.8	36.3	37.7
	8	50.5	36.4	40.1

Copepods were then evaluated. Any copepods still alive were quantified by manually counting them with a dissecting microscope. Copepods that were presumed to be dead (no movement after stimulation with a transfer pipette) were separated and allowed to rest at room temperature to ensure they did not re-animate. If re-animation occurred those copepods were accounted for; however, re-animation did not occur. Six replicates were performed for each time trial with each dish type. All statistical analyses were conducted in R (R Core Team 2017). Linear regression was performed to evaluate the role of predictor variables (water temperature, system temperature, container type, and trial time) on the proportion of copepods observed to be dead. New copepods were used for every trial to avoid pseudoreplication.

The above steps were followed for a trial with Chadian native copepods (*Mesocyclops* sp) using metal and plastic containers (Table 2). Copepods were exposed to the same heating system for 2, 4, and 6 h. Counting of copepods was performed in the same manner as described above.

Results

Trials using North American Macrocyclops *sp copepods*

Copepod loss (defined here as the mean proportion deceased) was greatest in the metal containers, but did occur to varying degrees in all container types regardless of time exposed to heated conditions (Figure 2). Metal containers resulted in the most copepods lost at each trial time, with complete mortality of all copepods by 4 h. Glass containers had the least amount of loss during the 2-h trial (23.2%) and 6-h trial (58.7%), and plastic containers had the least amount of copepods lost during the 4-h (39.2%) and 8-h (52.0%) trials. Loss of copepods in gourds was consistently higher than in the plastic and glass containers at all trial times.

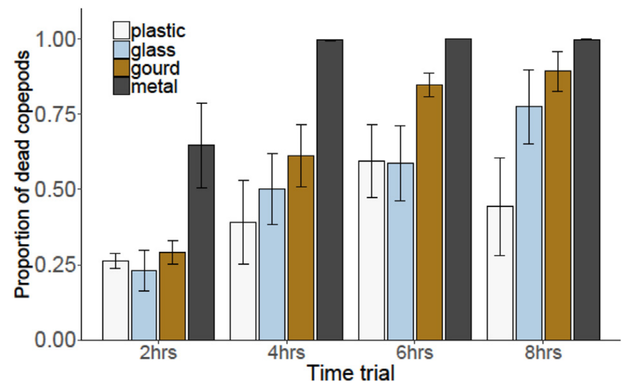

Figure 2. Copepod loss (mean proportion deceased) in container types according to time exposed to simulated Chadian temperature (34 °C).

Water temperature and time had significant effects on copepod survival, with increased water temperatures leading to increased mortality in copepods and longer trials resulting in higher quantities of copepods deceased in every container type (Tables 1 and 3). There was a significant association between trial duration and loss of copepods (Figure 2; Table 3).

Trials using Chadian Mesocyclops *sp copepods*

Copepod loss was greatest in metal containers, with complete mortality of copepods within 4–6 h. Metal containers also achieved higher temperatures more quickly than plastic dishes (Table 2).

Table 2
Chadian copepod survival study results. Each trial began with 10 cyclopoid copepods (*Mesocyclops* sp.) from Chad, Africa. Trials were duplicated with each container type.

		Trial length (h)	Number of copepods alive	Water temperature (°C)
Plastic	a	2	4	39.6
		4	2	39.6
		6	8	39.1
	b	2	4	41.3
		4	4	40.9
		6	7	39.2
Metal	a	2	5	42.0
		4	1	42.2
		6	0	39.1
	b	2	6	41.8
		4	1	41.4
		6	0	41.9

Discussion

The primary objective of this study was to evaluate copepod mortality in selected containers when exposed to Chadian ambient temperatures. The results showed that the container material and the length of exposure to high heat conditions were significantly associated with copepod mortality. The highest rate of copepod mortality was observed in metal containers, occurring as soon as 4 h. Interestingly, after prolonged exposures (8 h under constant heat), copepods were still alive in the glass, plastic, and gourd containers, indicating that these material types do a poor job at heating water to a thermal threshold capable of resulting in copepod mortality.

Table 3
Results of the roles of the different predictor variables using a linear model to analyze the effects of coefficients on North American *Macrocyclops*sp. copepod mortality in selected containers when exposed to Chadian ambient temperatures. p-Values < 0.05 (in bold) were considered to be significant.

Coefficient	Estimate	Standard error	Z-value	p-Value
Intercept	23.312087	1.169000	19.942	**<0.001**
System temperature	−0.001973	0.007059	−0.280	0.7798
Water temperature	−0.336662	0.016121	−20.883	**<0.001**
Humidity	−0.023397	0.004032	−5.802	**<0.001**
Room temperature	−0.328392	0.038528	−8.523	**<0.001**
Gourd	−1.140267	0.096473	−11.819	**<0.001**
Metal	−1.879366	0.094460	−19.896	**<0.001**
Plastic	0.139142	0.068184	2.041	**0.0413**
Trial time	−0.677799	0.028285	−23.963	**<0.001**

For provisioned water to be a risk, copepods have to both survive in the containers and be ingested by a dog. We partially selected the container types based on the types that are used in Chad, which primarily include metal, gourd, and black plastic water containers (Cleveland, personal communication). In most villages, the provisioned water containers are usually filled in the morning and then left for the day. Typically this water does not last the whole day due to evaporative loss or ingestion by dogs or other animals. As the study data showed that copepods can survive for at least 4 h, it is recommended that water be acquired from safe sources or filtered to remove copepods. In addition, this study was designed to mimic water placed in sunny areas; however, it is possible that copepods will survive longer in provisioned water placed in shady areas, because the water temperature may remain lower.

In a previous study, dogs were shown to ingest only low numbers of copepods in water when the density of copepods was similar to those noted in Chad (Garrett et al., 2020). These data, coupled with the high mortality of copepods in metal and gourd containers, suggest that provisioned water may only be a low risk factor for infection of dogs with *D. medinensis*. However, under certain conditions it could be an alternative source of infection. The challenge arises when dogs are tethered for suspect Guinea worm infection and are reliant upon provisioned water sources on a daily basis for a month or longer. For untethered dogs, their behavior may alter risk, but this needs further investigation (Hopkins et al., 2018), and to date no comprehensive peer-reviewed work has successfully investigated dog ecology and behavior. For example, when temperatures are at their highest, dogs tend to spend time wallowing in natural water bodies and likely drink from these water bodies instead of from the provisioned containers.

This study aimed to investigate the most suitable water containers to provision water to dogs during interventions such as proactive tethering in Chad, Africa. Variable survival rates were found for the different types of water container, but one initial limitation of the current study was the lack of native Chadian copepods. The copepods used in this study were a laboratory-raised *Macrocyclops* sp native to the United States and these may respond differently compared to native Chadian copepods. *Macrocyclops* sp. in the southeastern United States naturally exposed to high temperatures persist up to 40 °C (compared to Chad, Africa, which may average 33–45 °C monthly during the peak transmission season of March–June) (Rey et al., 2004). Therefore, the *Macrocyclops* sp. used here represent an appropriate surrogate for Chadian copepod species (Nematchoua et al., 2019). Nevertheless, to verify this concordance, and to further investigate potential variation in response to water temperature and dish type, a colony of Chadian copepods (*Mesocyclops* sp) was established and a trial using the metal and plastic containers exposed to the same heating system for 2, 4, and 6 h was conducted. No differences in copepod loss were noted between the different copepod species, and metal bowls led to complete mortality of copepods within 4–6 h (Table 2). Thus, we believe our results using North American copepods are applicable to Chad.

Understanding the various transmission pathways of Guinea worm is critical to the successful eradication of this parasite, especially as the numbers of dog and cat infections continue to increase. Between January and October 2019 alone, 1,885 dog *D. medinensis* infections were reported, representing an 85% increase in infections over the same period in 2018 (WHO Collaborating Center for Research Training and Eradication of Dracunculiasis, 2019). Until recently, waterborne transmission was considered to be the only route of infection, but there is now evidence that amphibians may serve as paratenic hosts and fish as transport hosts; although the importance of these routes is yet to be determined (Eberhard et al., 2016a; Eberhard et al., 2016b; Cleveland et al., 2019). For the eradication program to be successful, it is important to investigate all possible risk factors and the utility of individual interventions. The study data showed that under typical summer conditions in Chad, copepod survival in provisioned water was highly associated with the type of container used and the length of time that the water was exposed to high heat conditions. In summary, the use of metal containers for water provisioning created an environment that would be least likely to support a *D. medinensis* transmission event, as all copepods perished in a short time. Thus the use of metal containers could be a useful tool in assisting with the interruption of *D. medinensis* transmission among dogs.

Funding

This work was supported by The Carter Center; a full listing of Carter Center supporters is available at http://www.cartercenter.org/donate/corporate-government-foundation-partners/index.html. Support for Z. Eure was provided through the Population Biology of Infectious Diseases REU Site, a program funded by the National Science Foundation (Grant #DBI-1659683) Additional support was provided by the wildlife management agencies of the Southeastern Cooperative Wildlife Disease Study member states through the Federal Aid to Wildlife Restoration Act (50 Stat. 917) and by a US Department of the Interior Cooperative Agreement.

Ethical approval

Ethical approval was not needed for the work herein.

Conflict of interest

The authors have no conflicts of interest to declare.

Acknowledgements

The authors would like to thank Dr Robert Bringolf and Mr Bob Ratajczak for assistance and facilitation of laboratory space at the UGA Aquaculture Biotech Environmental Laboratory.

References

Awofeso N. Towards global Guinea worm eradication in 2015: the experience of South Sudan. Int J Infect Dis 2013;17:e577–82.

Cairncross S, Muller R, Zagaria N. Dracunculiasis (Guinea Worm Disease) and the eradication initiative. Clin Microbiol Rev 2002;15(2):223–46, doi:http://dx.doi.org/10.1128/CMR.15.2.223-246.2002.

Cleveland CA, Eberhard ML, Thompson AT, Smith SJ, Zirimwabagabo H, Bringolf R, et al. Possible role of fish as transport hosts for *Dracunculus* spp. larvae. Emerg Infect Dis 2017;23(9):1590–2, doi:http://dx.doi.org/10.3201/eid2309.161931.

Cleveland CA, Eberhard ML, Thompson AT, Garrett KB, Swanepoel L, Zirimwabagabo H, et al. A search for tiny dragons (Dracunculus medinensis third-stage larvae) in aquatic animals in Chad, Africa. Sci Rep 2019;9(1):375, doi:http://dx.doi.org/10.1038/s41598-018-37597-7.

Eberhard ML, Ruiz-Tiben E, Hopkins DR, Farrell C, Toe F, Weiss A, et al. The peculiar epidemiology of dracunculiasis in Chad. Am J Trop Med Hyg 2014;90(1):61–70, doi:http://dx.doi.org/10.4269/ajtmh.13-0554.

Eberhard ML, Yabsley MJ, Zirimwabagabo H, Bishop H, Cleveland CA, Maerz JC, et al. Possible role of fish and frogs as paratenic hosts of *Dracunculus medinensis*, chad. Emerging Infect Dis 2016a;22(8):1428–30, doi:http://dx.doi.org/10.3201/eid2208.160043.

Eberhard ML, Cleveland CA, Zirimwabagabo H, Yabsley MJ, Ouakou PT, Ruiz-Tiben E. Guinea worm (Dracunculus medinensis) Infection in a Wild Caught Frog, Chad. Emerging Infect Dis 2016b;22(11):1961–2, doi:http://dx.doi.org/10.3201/eid2211.161332.

Garrett KB, Box EB, Cleveland CA, Majewska AA, Yabsley MY. Dogs and the classic route of Guinea Worm transmission: an evaluation of copepod ingestion. Sci Rep 2020;1430(10), doi:http://dx.doi.org/10.1038/s41598-020-58191-4.

Hopkins DR, Ruiz-Tiben E, Eberhard ML, Roy SL, Weiss AJ. Progress Toward Global Eradication of Dracunculiasis, January 2016–June 2017. MMWR Morb Mortal Wkly Rep 2017;66:1327–31.

Hopkins DR, Ruiz-Tiben E, Eberhard ML, Weiss A, Withers Jr. PC, Roy SL, et al. Dracunculiasis Eradication: are we there yet. Am J Trop Med Hygiene 2018;99 (2):388, doi:http://dx.doi.org/10.4269/ajtmh.18-0204.

Nematchoua MK, Orosa JA, Reiter S. Climate change: variabilities, vulnerabilities and adaptation analysis – a case of seven cities located in seven countries of Central Africa. Urban Climate 2019;29, doi:http://dx.doi.org/10.1016/j.uclim.2019.100486.

Rey JR, O'Connell S, Suárez S, Menéndez Z, Lounibos LP, Byer G. Laboratory and field studies of Macrocyclops albidus (Crustacea: Copepoda) for biological control of mosquitoes in artificial containers in a subtropical environment. J Vector Ecol 2004;29(1):124–34.

Thiele EA, Eberhard ML, Cotton JA, Durrant C, Berg J, Hamm K, et al. Population genetic analysis of Chadian Guinea worms reveals that human and non-human hosts share common parasite populations. PLoS Negl Trop Dis 2018;, doi:http://dx.doi.org/10.1371/journal.pntd.0006747.

WHO Collaborating Center for Research Training and Eradication of Dracunculiasis. Guinea Worm Wrap-Up #264, December 9, 2019. Atlanta: Centers for Disease Control and Prevention (CGH); 2019.

WHO Collaborating Center for Research Training and Eradication of Dracunculiasis. Guinea Worm Wrap-Up #265, January 13. 2020. Atlanta: Centers for Disease Control and Prevention (CGH); 2020.