Dogs and the classic route of Guinea Worm transmission: an evaluation of copepod ingestion

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Dracunculus medinensis, the causative agent of Guinea worm disease in humans, is being reported with increasing frequency in dogs. However, the route(s) of transmission to dogs is still poorly understood. Classical transmission to humans occurs via drinking water that contains cyclopoid copepods infected with third stage larvae of D. medinensis, but due to the method of dog drinking (lapping) compared to humans (suction and/or retrieval of water into containers), it seems unlikely that dogs would ingest copepods readily through drinking. We exposed lab raised beagles to varying densities of uninfected copepods in 2 liters of water to evaluate the number of copepods ingested during a drinking event. We confirmed dogs can ingest copepod intermediate hosts while drinking; however, low numbers were ingested at the densities that are typically observed in Chad suggesting this transmission route may be unlikely. Overall, the relative importance of the classic transmission route and alternate transmission routes, such as paratenic and transport hosts, needs investigation in order to further clarify the epidemiology of guinea worm infections in dogs.

Guinea worm disease (GWD), caused by Dracunculus medinensis, is a painful and debilitating disease that is historically widespread in humans among sub-Saharan Africa and southern Asia. The international campaign to eradicate GWD has been extremely successful and has resulted in a decrease of >3.5 million human cases annually in 21 countries to only 49 human cases in 2019 (43 in Chad, 4 in South Sudan, 1 in Angola, 1 in Cameroon); however, infections of animals occur in four countries (Chad, Ethiopia, Mali, and Angola)¹. The vast majority of these animal infections are from Chad (n = 1,901) and in domestic dogs in Chad (n = 1,855)¹. In Chad, there are limited numbers of human infections despite the significant increase in the number of infected domestic dogs and domestic cats, suggesting alternative transmission pathways may be involved in some animal infections²,³. Recent data indicates that amphibians can be paratenic hosts and fish can be transport hosts, possibly contributing to this unusual epidemiology⁴–⁶. Two recent genomic studies showed that the D. medinensis derived from humans and animals are the same species and part of the same population of worms⁷,⁸. Thus, animal infections are a major concern for the eradication campaign as they can contaminate water sources, which thereby could increase the risk of human or additional animal infections².

In humans, transmission of D. medinensis is considered to occur through the ingestion of infected copepods in contaminated drinking water. Effective control strategies were developed and implemented to break this transmission route to humans. However, the standard control strategies used for humans (e.g., filtering water, using safe water sources) cannot easily be applied to animal populations. Currently, given the unusual epidemiology compared to typical human infections, there is debate on the source of dog infections²,³,⁹. They can become infected through ingestion of infected copepods but could also become infected through ingestion of infected amphibian paratenic or fish transport hosts. In a first step to investigating the relative importance of these different transmission routes, we conducted this study to determine if dogs ingest copepods during a drinking event and, if so, to quantify ingestion at different copepod densities. When dogs lap, liquid adheres to the tongue and is ingested against gravity as opposed to suction drinking, the creation of a vacuum to ingest water, used by humans or some animals (e.g., baboons); therefore, dogs may not readily ingest copepods⁹.
of partial cytochrome oxidase subunit 1 (COI) gene sequence as described 11. In Africa, species of
sp. through morphology and analysis
Georgia, Athens, GA, USA. Copepods were identified as a
Macrocyclops
from Bishop, GA, USA, and housed at the Aquatic Biotechnology and Environmental Lab at the University of
the 50 copepods group, 7 for the 100 group, 41 for the 500 group, and 96 for the 1000 group (Fig. 1b).

Dogs were fasted from water 12 hrs prior to each trial. During each drinking event, dogs were presented
with two liters of dechlorinated water spiked with one of five copepod density groups (0, 50 [25/L], 100 [50/L],
500 [250/L], and 1000 [500/L]) (Table 1). Copepods were lab-raised from a
initial group of copepods collected from Bishop, GA, USA, and housed at the Aquatic Biotechnology and Environmental Lab at the University of
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Mesocyclops, Thermocyclops, and Macrocyclops
are believed to be the most important intermediate hosts for
D. medinensis12.

Each dog was exposed to the water within their regular enclosures for one hour to allow for natural lapping
conditions. Experimenters left the room during this period to avoid distracting the dogs. After an hour, water was
removed, and the catch basin rinsed to recover any copepods that may have adhered to the basin wall. Remaining
copepods were quantified and the amount of water consumed was recorded. We ran three replicates of the drinking
trials, yielding a total of 60 observations. Each dog was exposed to one of the five different copepod concentra-
tions weekly for five weeks. All experimental methods were reviewed and approved by the University of Georgia's
Institutional Animal Care and Use Committee (A2016 11-004) and were performed in accordance with relevant
guidelines and regulations.

We tested whether the number of consumed copepods was influenced by initial copepod concentration. We fit a
generalized linear mixed effects model (GLMM; lme4 package13) with negative binomial errors. The initial
concentration of copepods was the explanatory variable and the offset was volume of water ingested to account
for variation in the amount of water ingested by each dog. To account for repeated measures of each dog, we
included dog identification and replicate number as random effects. We used R programming software for the
statistical analyses14.

Table 1. Design of the dog drinking trial for a single replicate.

|       | Dog 1 | Dog 2 | Dog 3 | Dog 4 |
|-------|-------|-------|-------|-------|
| Tuesday | 0     | 1000  | 50    | 100   |
|       | 1000  | 0     | 50    | 1000  |
| Dog 2 | 0     | 50    | 100   | 0     |
|       | 100   | 0     | 50    | 100   |

Methods
To examine whether dogs ingest copepods during a drinking event, four lab-raised beagles (Ridglan Farms,
Inc.) of the same age were individually housed in a climate-controlled (70 °F, 21°C) facility and provided food
and water ad libitum (except for the 12 hrs before being provided copepod-spiked water samples wherein water
was withheld, but food was made available). Lapping trial containers consisted of a 2.5L square glass container
(Pyrex®) attached to a metal 2-in deep catch basin with Velcro®. The catch basin caught any water or copepods
that were splashed out but not consumed during a drinking event. Lapping trial containers were secured to the
wall of the dog enclosure to reduce splashing and spilling.

Dogs were fasted from water 12 hrs prior to each trial. During each drinking event, dogs were presented
two liters of dechlorinated water spiked with one of five copepod density groups (0, 50 [25/L], 100 [50/L],
500 [250/L], and 1000 [500/L]) (Table 1). Copepods were lab-raised from a
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Ethical approval and informed consent. All experimental methods were reviewed and approved by
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vant guidelines and regulations.

Results
Copepod consumption varied somewhat among individuals (Fig. 1a), and statistical analysis showed that the
number of copepods consumed was positively influenced by the initial number of copepods presented to a dog
(z = 6.6, p < 0.001). The average number of copepods consumed per copepod concentration were: 3 copepods for
the 50 copepods group, 7 for the 100 group, 41 for the 500 group, and 96 for the 1000 group (Fig. 1b).

Discussion
Our data indicate that under experimental conditions, dogs may ingest copepods during a drinking event. Thus, it
is theoretically possible that enough infected copepods could be ingested to result in dog infections if the copepod
infection rate was high enough. We also found that higher densities of copepods led to higher ingestion rates.
In Chad, copepod density varies seasonally and between water-bodies. A recent survey found that the average
annual number of copepods in four ponds in Chad was 100–200 copepods/L with periodic and short-term spikes
of 400–700 copepods/L, primarily in the summer months (T. Moundai, unpublished data); however, additional
variation may be present in other regions of Chad. Our highest density tested (1000 [500 copepods/L]) represents
the highest naturally observed densities in Chad, while the lower and middle doses (100 [50/L] and 500 [250/L])
represent more frequently detected densities. If numbers of copepods ingested under natural conditions is sim-
ilar to our experimental data, numerous drinking events from contaminated water bodies may be necessary for
Dracunculus transmission to occur. It is important to note the prevalence of infected copepods in a contaminated
pond is poorly understood but is presumed to be relatively low based on past studies (range of 0.5–33.3%, average
of 5.2%)15–17. Also, because both a female and male worm are required to complete the life cycle, and copepods
infected with more than one Dracunculus L3 are likely to die18, multiple infected copepods must be ingested.
Although it is theoretically possible to have a single male and single female larvae mature, numerous experi-
mental studies have shown that many ingested larvae fail to mature in definitive hosts; thus multiple infected
copepods likely have to be ingested14,15. At copepod densities of 50 [25/L] and 100 [50/L], 3–7 copepods were
consumed on average (Fig. 1b); thus the likelihood of a dog ingesting multiple infected copepods during the two to three week time period in which the copepods are infected with mature L3’s, and thus infections to a definitive host may be low. However, it cannot be ruled out or minimized, especially within the context of eradication. Other considerations must be addressed during future studies, ideally under field conditions. Our dogs were maintained indoors under climate-controlled conditions (21 °C), whereas there are more extreme temperatures (40 °C to 43 °C) during the peak transmission season in Chad. To partially account for this, water was withheld from dogs for 12 hrs (maximum allowed). Also, our copepods were collected from Georgia, USA which may behave differently than Chadian copepods. Relatively small glass dishes were used in this study; however, wild copepods likely have more opportunities to flee from dogs within larger water volumes. Finally, under natural conditions, copepods may flee into substrate of water-bodies, which is especially known to occur during daylight hours19. Another important consideration is that dogs in Chad will often wade and lie in water-bodies leading to disruption of copepods, which could influence the likelihood of ingestion. It is possible that this disruption could decrease risk of ingestion as copepods flee the disturbance. This is assuming that the behavior of infected copepods is similar to uninfected copepods. One study by Onabamiro (1954) found that infected copepods were more sluggish than uninfected copepods; however, a large number of parasites were provided to copepods so many likely had multiple larvae which could have impacted their behavior more than what typically occurs in nature (copepods infected with a single larva)16,20.

In conclusion, despite our small sample size, our data indicate that dogs can ingest relatively few copepods while drinking, but there are still many factors to investigate to determine the primary transmission route(s) of *D. medinensis* in the remaining GWD-endemic countries. Other possibilities include ingestion of amphibian paratenic hosts or fish transport hosts2,4-6,15. Currently, there are many interventions in place to minimize transmission risk including: tethering of infected dogs, treatment of potentially contaminated water bodies with Abate®, and the burning or burial of fish entrails, but dog infections continue to occur suggesting that improved adherence to interventions or new interventions are necessary to interrupt transmission. Continued evaluation of transmission routes, in the field and theoretically, may help refine or develop interventions.

**Disclaimers.** The opinions expressed by authors contributing to this journal do not necessarily reflect the opinions of the institutions with which the authors are affiliated.
Data availability
The datasets generated during this study are available from the corresponding author upon reasonable request.

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Author contributions
All authors made substantial contribution to the conception, design, acquisition, analysis, and/or interpretation of the data for this study. All authors were involved in drafting of the manuscript and approved of the submitted version and edits. All authors agree to be personally accountable for their contributions. Statement K.B.G. wrote the main manuscript text. K.B.G., E.K.B., and C.A.C. conducted the experiment. A.A.M conducted the statistical analyses and prepared Figure 1. All authors provided edits to the manuscript. M.J.Y. was the principal investigator for the experiment. K.B.G. is a research professional at the Southeastern Cooperative Wildlife Disease Study at the University of Georgia. Her research interests include the ecology of infectious diseases, emerging infectious disease, and wildlife management.

Competing interests
The authors declare no competing interests.

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