Ticks and Tick-borne Diseases 12 (2021) 101780

Contents lists available at ScienceDirect

Ticks and Tick-borne Diseases

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

Original article

Are Virginia opossums really ecological traps for ticks? Groundtruthing laboratory observations

Cecilia Hennessy *, Kaitlyn Hild

Division of Math and Sciences, Eureka College, Eureka, IL, United States

ARTICLE INFO

Keywords:
Didelphis virginiana
Diet analysis
Ecological trap
Scientific communication
Tick
Vector
Virginia opossum

ABSTRACT

Virginia opossums (*Didelphis virginiana*) are a common synanthrope in North America, and serve as host to many species of ectoparasites. Research on captive Virginia opossums estimated that opossums eat, on average, 5500 larval ticks (Acari: Ixodidae) per week. To investigate this apparent preference exhibited by opossums for ingesting ticks, we comprehensively analyzed stomach contents of 32 Virginia opossums from central Illinois. Using a dissecting microscope, we searched the contents exhaustively for ticks and tick body parts, without sieving or pre-rinsing the stomach contents. We did not locate any ticks or tick parts in the stomach contents of Virginia opossums. We also performed a vigorous literature search for corroborating evidence of tick ingestion. Our search revealed 23 manuscripts that describe diet analyses of Virginia opossums, 19 of which were conducted on stomach or digestive tract contents and four of which were scat-based analyses. None of the studies identified ticks in their analyses of diet items. We conclude that ticks are not a preferred diet item for Virginia opossums. Considering that wildlife unconditioned to laboratory conditions may exhibit non-typical behaviors, we recommend that lab-based studies of wildlife behavior be groundtruthed with studies based in natural conditions.

Introduction

Zoonotic pathogens are disease-causing agents that can be transmitted from animals to humans. In comparison to non-zoonotic pathogens, zoonotic pathogens are twice as likely to be the cause of emerging disease threats (Taylor et al., 2001). Wildlife species that serve as reservoirs for diseases and the ectoparasites that serve as vectors between wildlife and humans must be monitored to assess potential risks of zoonotic outbreaks (Polley, 2005). The Virginia opossum (*D. virginiana;* hereafter "opossum") is a synanthropic (meaning it thrives in human-dominated ecosystems), opportunistic generalist and scavenger (Gardner, 1982). In North America, the opossum is is a host for myriad tick species such as the dog tick (*Dermacentor variabilis*), blacklegged tick (*Ixodes scapularis*), lone star tick (*Amblyomma americanum*), groundhog tick (*I. cookei*), and the rabbit tick (*Haemaphysalis leporispalustris*), among others (Bezarra-Santos et al., 2021; Whitaker et al., 1977). Ticks that are specialists on other species, such as the groundhog tick and the rabbit tick, are often found on opossums, likely transferring while the opossums feed on the ticks' original host. This unusual trait earned the opossum the moniker of "carrier host" by Wenzel and Tipton (1966). Opossums' tick burdens vary across regions and seasons: opossums in southwestern Tennessee carried on average 5.03 ± 7.88 dog ticks (Kollars, 1993), a study in Nebraska found on average 14.33 adult dog ticks per opossum (Durden and Richardson, 2013), whereas a researcher in upstate New York found that only seven of 200 opossums had ticks attached (Hamilton, 1958); of those seven the burden ranged from one to eight *I. cookei* per opossum. Fish and Daniels (1990) captured six opossums in southern New York, all of which were infested with *I. scapularis* larvae, with a mean of 54 ticks per host.

Although opossums are inefficient at spreading rabies (Barr, 1963), they are not zoonotically neutral. Opossums have the potential to serve as reservoirs for tick-vectored diseases that pose risks to humans, such as *Babesia microti* (a blood-borne parasite which causes babesiosis; Yabsley and Shock, 2013), *E. chaffeensis* (which causes ehrlichiosis; Lockhart et al., 1997), *Rickettsia rickettsii* (which causes Rocky Mountain Spotted Fever; Schumacher et al., 2016), and *Borrelia burgdorferi* sensu lato (which causes Lyme disease; Fish and Daniels, 1990). Opossums serve as the definitive host for *Sarcocystis neurona,* which causes equine protozoal myeloencephalitis (EPM), a disease that can be fatal to horses (*Equus ferus caballus*; Fenger et al., 1995). Recent research shows that

* Corresponding author.
E-mail address: cecilia.hennessy@gmail.com (C. Hennessy).

https://doi.org/10.1016/j.ttbdis.2021.101780
Received 5 January 2021; Received in revised form 4 June 2021; Accepted 28 June 2021
Available online 15 July 2021

climate change is influencing northward range expansion in opossums, a trend that may exacerbate the spread of disease (Walsh and Tucker, 2018). Due to the diversity of disease vectors they host, their reservoir capacity, and their expanding range, opossum populations warrant monitoring programs and zoonotic prevention measures. Therefore, it is critical for researchers to have a clear understanding of opossum-tick relationships in order to accurately gauge public health threats from this synanthropic species.

Recent research suggests that opossums can be expected to decrease zoonotic risk in an ecosystem based on the premise that opossums consume more ticks than they disperse, thereby serving as an ambulatory ecological trap for vectors of diseases. Keesing et al. (2009) claimed that Virginia opossums consume, on average, upwards of 5500 larval ticks per week during the larval activity peak. The authors calculated this estimate by placing a known amount ($n = 100$) of larval blacklegged ticks (*Ixodes scapularis*) on five captive Virginia opossums and then counting how many fell off over the course of four days. The 100 larval ticks were only placed on the opossums once, which is very different from the type of ongoing exposure opossums would experience in their natural settings. The authors found that, on average, only 3.5 larval ticks fell off each opossum having ingested a blood meal, and the rest could not be located in the cage set-up, prompting the authors to assume that the ticks were eaten by the opossums while self-grooming. Based on their assumption that average larval tick burdens of opossums in upstate New York are 199 $\pm$ 90, the authors stated "the vast majority (96.5%) of larval ticks that encounter an opossum and attempt to feed are apparently consumed. Working backwards, during any given week in the larval activity peak, each opossum must host more than 5500 larval ticks to produce 199 that successfully feed." (pg. 3913, Keesing et al., 2009). According to the methods in the manuscript and verified by the lead author (F. Keesing, personal communication), the authors did not remove any ticks from the opossums on intake nor did they check the opossums for remaining ticks before releasing them into their habitats. The researchers assumed that any tick that did not achieve repletion within four days was ingested by the opossum. In two lay publications discussing these findings (Miller, 2014; Lipske, 2015), co-author Richard Ostfeld described verifying tick ingestion by performing a scat analysis on the captive opossums, a procedure that was not described in the original paper and did not take place systematically (F. Keesing, personal communication).

This estimate of 5500 ingested larval ticks per week has been used as an assumption in zoonotic models (e.g., Levi et al., 2016). It has been widely disseminated via trade publications (e.g., Black, 2020), lay publications (e.g., Willson; Edmonds, 2019; Connolly, 2020, Hetzler), institutionally affiliated blogs (e.g., Feldner, 2019), as well as in podcasts (e.g., https://podcasts.apple.com/us/podcast/episode-169-awesome-o-possum/id1311040782?*i* = 1000479207908) and memes on social media (Fig. 1). The concept that opossums are "hoovering up ticks" (Lipske, 2015) has inspired some members of the public to attract opossums to their yards (e.g., Willson) as a form of zoonotic disease risk reduction. This is problematic, because methods of attracting opossums to human residences would also attract species, such as feral cats (*Felis catus*), raccoons (*Procyon lotor*), rats (*Rattus* spp.), and mice (*Peromyscus* and *Mus* spp.) that serve as vectors for toxoplasmosis, rabies, murine typhus, among other zoonotic diseases (Mackenstedt et al., 2015). Moreover, opossums themselves are vectors for other diseases, as reviewed above. Many members of the scientific community and the public have been seduced by the claim that opossums are actively seeking ticks as diet items, and because this claim came from peer-reviewed scientific literature, it has been largely accepted as "fact".

To test the premise that Virginia opossums are eating 5500 ticks per week, we investigated tick ingestion by opossums with a bifurcated approach. First, we investigated stomach contents of wild-sourced Virginia opossums with thorough methods, specifically seeking evidence of

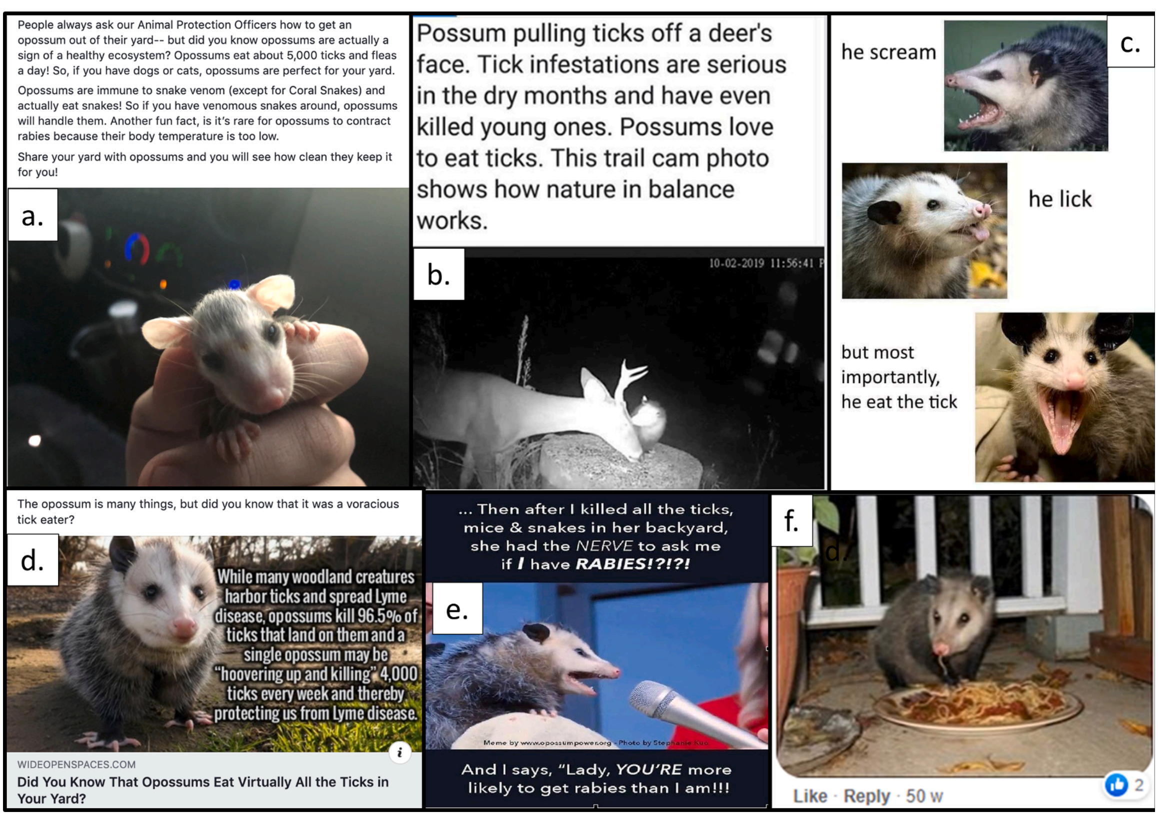

Fig 1. Sampling of memes collected from social media that demonstrate the belief that Virginia opossums (*Didelphis virginiana*) perform a substantial ecosystem service by actively consuming ticks. Panel 1f demonstrates an instance of feeding opossums to attract them to residences. Used with permission when possible, but original author of memes was not possible to determine in every case (refer to Hanganu-Bresch, 2017).

tick ingestion. Second, we investigated the scientific literature for all diet analyses performed on Virginia opossums. We predicted that Virginia opossums did consume ticks, but not at the high rates estimated.

Materials and methods

Stomach content analysis

We obtained 32 specimens from sites in central IL, including Woodford, Tazewell, and Peoria counties. This region is dominated by row crop agriculture with deciduous riparian forests and extensive suburban development. There are also native prairie remnants and substantial urban areas, including the cities of Peoria, Metamora, Washington, and Eureka. Verified tick species found on mammals in the sampling area include: blacklegged tick, lone star tick, dog tick, and winter tick (*D. albipictus*; Cortinas and Kitron, 2006, see also Eisen et al., 2016). Ongoing epidemiological reporting (Illinois Department of Public Health, 2019) in the three counties shows rates of Lyme disease per 100,000 persons as 4.1 (Woodford), and 2.1 (Tazewell), and 2.8 (Peoria; Fig. 2). Heartland virus, a recently identified disease that is potentially fatal to humans, has been detected in lone star tick populations in other counties in the central Illinois region (Tuten et al., 2020). As ticks in Illinois are generally most active in the months June-August (Kitron et al., 1991), we focused the majority of our collection in those summer months (see Table 1 for seasonal distribution of sampling).

Opossums collected for this study were live-trapped and euthanized by a Class A Nuisance Wildlife Control Operator (NWCO) or collected incidentally as roadkill under an Illinois DNR scavenging permit. One recently deceased opossum was located at a forested park, cause of death unknown. No opossums were trapped or euthanized for the purposes of this study. The specimens were checked for age, sex, and presence of ticks, then stored in a freezer until prepared for examination. Previous studies have shown that stomachs alone are sufficient for a comprehensive analysis for diets of opossums, as analyzing the rest of the gastrointestinal tract does not typically add new information (Hamilton, 1951). Therefore, using cotton string, we tied off stomachs approximately 2 cm above the esophageal sphincter and approximately 2 cm below the duodenum to retain contents. Stomachs were stored in 70% non-denatured ethanol at room temperature. Proper health and safety precautions were taken at every stage in the investigation.

For analysis, we opened the stomach longitudinally with scissors to

Table 1
Presence-absence information regarding items found in 32 analyzed stomachs of Virginia opossums (*Didelphis virginiana*) collected as roadkilled specimens or live-trapped in central Illinois, counties Woodford, Tazewell, and Peoria, 2017–2019.

Items	Spring: March-May	Summer: June-Aug.	Fall: Sept.-Nov.	Winter: Dec.-Feb.
Stomachs Analyzed	4	20	6	2
Invertebrates				
Siphonaptera/Flea	—	2	—	—
Coleoptera/Beetle	1	7	1	—
Annelida/ Earthworm	—	—	1	—
Diptera/Fly	—	3	3	—
Lepidoptera/ Caterpillar	—	1	—	—
Nematodes	2	10	5	2
Orthoptera/ Grasshopper	—	1	1	—
Ixodida/Tick	—	—	—	—
Vertebrates				
Amphibian	—	1	—	—
Bones	—	—	1	—
Feathers	—	4	1	—
Fur	2	13	3	—
Small mammals	—	2	—	—
Skin	—	—	3	—
Teeth	—	1	2	—
Plant Matter				
Flower	—	1	—	—
Grass	—	6	1	—
Leaf	—	5	2	—
Roots	1	7	—	—
Seed	—	5	—	—
Wood	—	3	—	—
Garbage/ Anthropogenic				
French Fry	—	1	—	—
Glass	—	—	2	—
Plastic	—	2	1	—
Digestive Matter				
Gelatinous mass	1	2	2	—
White bits of chyme	—	3	—	—

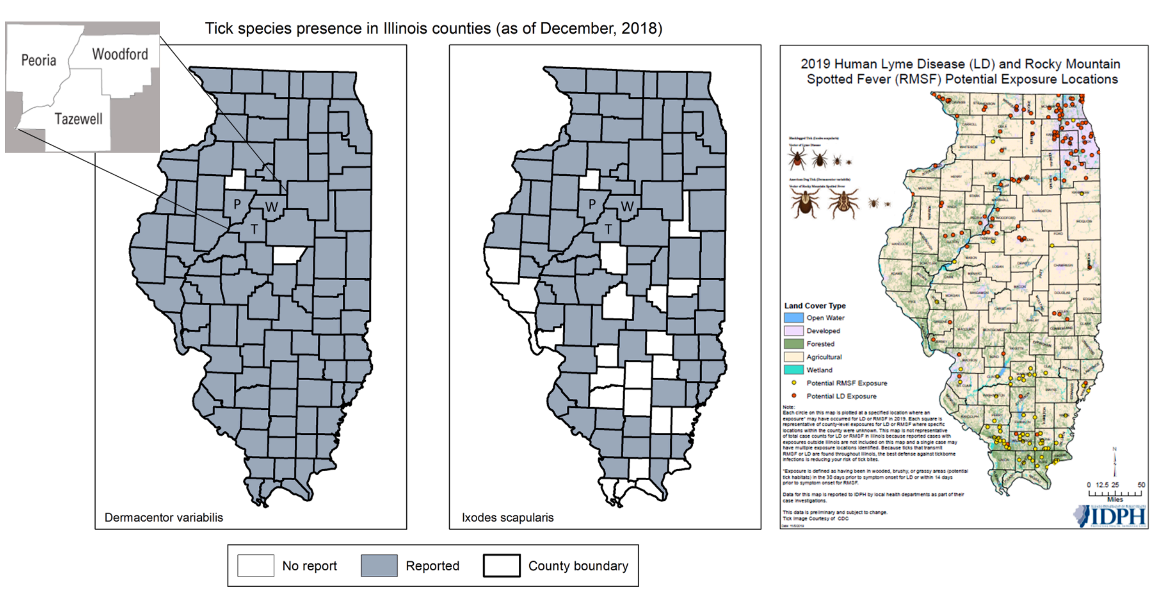

Fig 2. Detection maps of *Dermacentor variabilis* and *Ixodes scapularis* in Illinois, and mapped incidence of tick-transmitted Lyme disease in Illinois. Note the sample area of Peoria (P), Tazewell (T), and Woodford (W) counties (reprinted with permission; Illinois Department of Public Health, 2019).

expose all contents. A metal spatula was used to scrape out contents into a plastic tray. From this, small amounts of matter were transferred into a Petri dish for inspection with a dissecting microscope (LabOMed Luxeo 2S, Los Angeles CA, USA). A constant, slow agitation with a dissecting probe while viewing through the scope was necessary to expose items in the turbid chyme. We added water as needed to expose items for further analysis. Items that required further analysis were transferred to microscope slides and examined with a compound microscope (Nikon SE, Nikon, Garden City, NJ, USA). Items were compared visually with images from a comprehensive arthropod reference guide with detailed color photographs (Marshall, 2006). Preserved specimens of an adult and a larval tick (*Ixodes scapularis*) from a biological supply company were available to be used as reference items. Once inspected and identified, contents were returned to the original specimen jar, along with the stomach. This process was undertaken for the entirety of stomach contents in each specimen, not just a sample of each.

Literature review

To determine if other studies found anything similar in regards to tick consumption by Virginia opossums, we performed an intensive literature search on opossum ecology and diet analyses. We used Google Scholar, EBSCO, and JSTOR search engines with a variety of combinations of search terms, including “Virginia opossum”, “D. virginiana”, “Diet”, “Stomach”, among others, to discover relevant publications. We also tracked down publications cited as references in other publications. Note that some of the older studies may have been of locations that were not verified at that time as having established tick populations (per Eisen et al., 2016).

Results

Stomach content analysis

We collected 32 (12 adult females, 10 adult males, 6 juvenile males, 4 juvenile females) opossum carcasses in all seasons from 2017 to 2019 (Table 1). Juveniles were past the joey stage, yet were not full-sized or sexually mature. Ticks were embedded on several carcasses that were live-trapped and euthanized by the licensed NWCO shortly before carcasses were placed into a freezer (mean number of ticks per opossum: 6.1, range 0–18). We found very few ticks on the roadkilled specimens (mean = 2.1, range = 0–7); however, roadkilled specimens do not generally provide an accurate estimate of tick burden, as most ectoparasites will detach from their host soon after the host’s death (Andrew and Norval, 1989). In our exhaustive analysis of stomach contents (n = 32; Table 1; Fig. 3), we did not locate any ticks in adult, nymph, or larval form. We identified two individual fleas (*Ctenocephalides* spp.; Fig. 3c). There were many arthropod items, but none of them were from Class Arachnida, which includes ticks and mites of the Subclass Acari. One stomach contained a minimally masticated short-tailed shrew (*Blarina brevicauda*; Fig. 3d). We found abundant nematodes, likely of species *Turgida turgida*, based on appearance and reported rates of infestation (Krueger et al., 2016). Two opossum stomachs, both collected in winter, were empty except for nematodes.

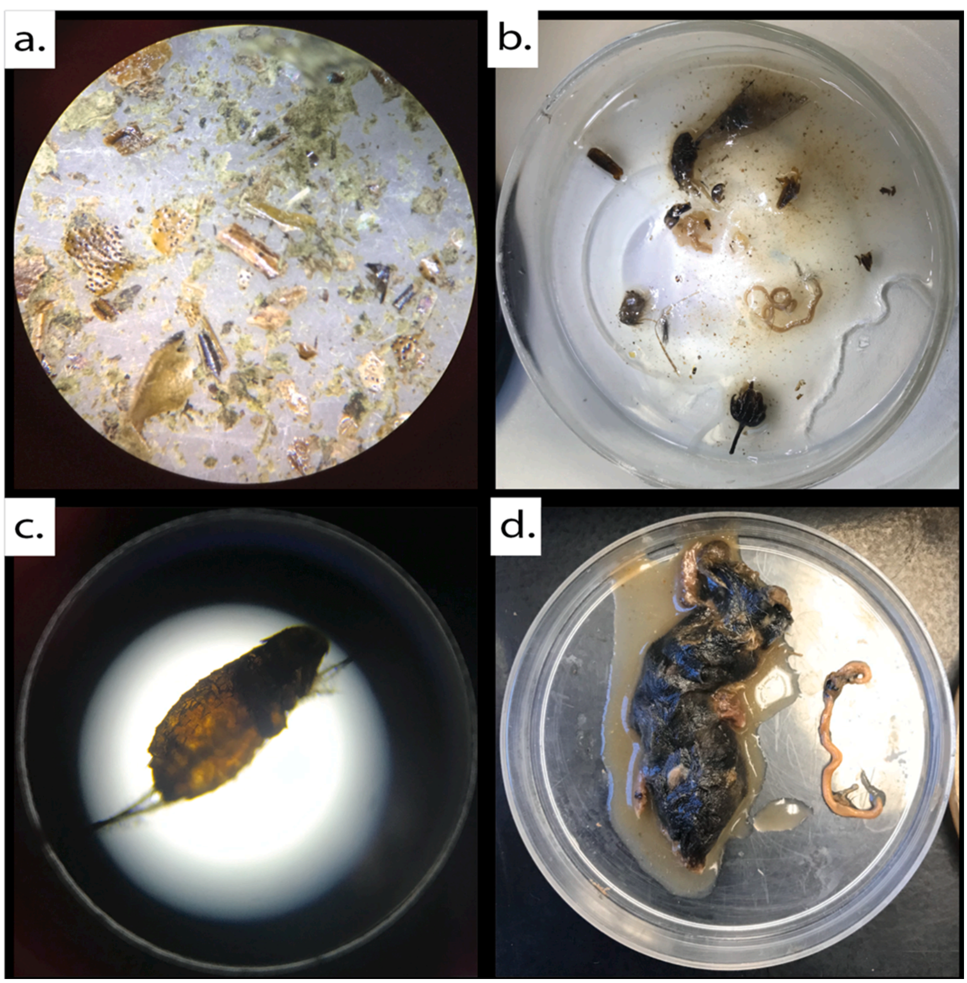

Fig 3. Four examples of items found in Virginia opossum (*Didelphis virginiana*) stomachs, including plant matter (a; 10x), plant matter and nematodes (b), flea (c, *Ctenocephalides* spp., 20x) and a minimally masticated short-tailed shrew (d, *Blarina brevicauda*) with nematode.

With regard to methodology, we found it labor intensive and time consuming to identify items in the turbid chyme of the stomach contents. Therefore, we recommend that researchers performing stomach analyses consider sieving stomach contents and rinsing before attempting to identify items, using sieves of appropriate sizing (Table 2). We recommend saving contents after analyzing, so that re-analysis is possible, if needed. We also recommend storing contents in 70% non-denatured ethanol (which has not had methanol added to it; sometimes labeled "molecular grade") to facilitate genetic analyses if warranted. Methanol damages DNA and renders downstream genetic analysis impossible.

Literature review

We located 23 published sources that describe diet items of Virginia opossums in detail, 19 of which were conducted on stomach or entire digestive tract analysis, and four of which were conducted on scat (Table 2). Natural history investigations often included stomach content analysis using various methods. For example, several previous studies into opossum diets included a rinsing step (or two, *sensu* Hopkins and Forbes, 1980), which could potentially wash out tiny particles such as tick larvae and tick body parts (Table 2). One study was performed in the field, without use of microscopes (Audubon and Bachman, 1851). Scat analyses were also reported; however, this method is not as accurate as stomach content analysis, due to potential misidentification of found scat and also due to digestive breakdown of many types of food items (Hamilton, 1951). Only one previous investigation was undertaken to determine the importance of a specific diet item: earthworms (Dexter, 1951). In none of these sources were ticks, in any life stage (egg, larva, nymph, or adult), listed as an item that was found in the digestive tracts or scat content. Fleas and mites are occasionally listed (e.g. Hopkins and Forbes, 1980), which were assumed to have been consumed during grooming. Grooming was also the likely source of opossum fur that was found in the stomachs and digestive tracts in all the studies, although occasionally opossum skin and fur were identified together, indicating that the opossum had consumed the remains of another opossum (e.g. Reynolds, 1945). All studies showed a preponderance of insects and other invertebrates in the diets of opossums, regardless of location. For example, (Lay, 1942) showed that insects comprised 45% of the diet of specimens ($n = 16$), and (Dearborn, 1932) found that 16.7% of the diet of specimens ($n = 40$) was composed of insects. However, Hopkins and Forbes (1980) examined 77 stomachs from opossums in Portland, Oregon, and concluded that insects only comprised 3% of the marsupial's diet, possibly due to lower incidence of insects in urban areas. Some variation in diet is evident across seasons, with insects typically comprising a larger fraction during the months of insect activity. When considering the volume of invertebrate content, Orthoptera species and Coleoptera species comprised the largest fraction, rivaled in some individual cases by earthworms (*Lumbricus terrestris*; Dexter, 1951) or terrestrial snails (Reynolds, 1945). Arachnids were listed as trace items in Blumenthal and Kirkland (1976), but the authors did not indicate whether the arachnids were ticks, mites, spiders, or harvestmen. A few mites were found and identified to genus by Hopkins and Forbes (1980). Possibly the most thorough account is given by Hamilton (1958), who investigated 461 stomachs and identified each arthropod fragment to family, if not to genus. Whitaker et al. (1977) is also notable for identifying each arthropod item in 66 opossum stomachs to family. Species accounts (McManus, 1974; Gardner, 1982) give broad perspectives on the opossum's diet based on reviews of 8 and 22 published resources, respectively. Not a single publication recognized ticks as a component in the diet of this species, even as a trace item.

Discussion

The conclusion by Keesing et al. (2009) that opossums consume 96.5% of the ticks on their bodies via grooming was not supported by the published literature concerning the ecology of this species nor by our examination of 32 stomachs specifically seeking evidence of ticks in the opossum diet. Keesing et al. (2009) did not state whether the grooming behaviors necessary to remove the larval ticks were observed in the lab, but they assumed those behaviors must have occurred because the larval ticks were not collected in the cage set-up. We question whether there could have possibly been another way for the placed larval ticks to have escaped researchers' detection. The first consideration is repletion period. Martins et al. (2012) performed a laboratory experiment and found that larval ticks (*Amblyomma ovale*) can feed on white-eared opossums (*D. albiventris*) for up to 10 days, with an average feeding period of 5 ($\pm$0.9) days. Larvae of another ixodid species, *D. andersoni*, feed on hosts 3–6 days on average before achieving repletion (Apanaskevich and Oliver, 2014). Knight et al. (1978) found that ticks in the

Table 2
Peer-reviewed studies of Virginia opossum (*Didelphis virginian*) diet analyses. None of these studies reported finding ticks (Suborder: Ixodida) when investigating digestive tracts (which include stomachs), stomachs only, or scats. Capture methods include trapping (T), incidentally encountered (I), and hunted (H), with reported sample sizes (n). Some investigations included a second method of diet analysis (Method 2). NR stands for not reported.

Publication	Capture	Method 1	n 1	Sieved (size)	Method 2	n 2	Location	Months Taken
Audubon and Bachman, 1851	NR	Stomach only	NR	NR			Virginia	NR
Blumenthal and Kirkland, 1976	T	Digestive tract	62	Yes (1 mm)			Pennsylvania	Mar-Jan
Brocke, 1970	I	Digestive tract	20	NR			Michigan	Jan-Mar
Dearborn, 1932	T	Stomach only	40	No			Michigan	Nov, Mar, July-Aug
Fitch and Sandidge, 1953	I	Scat	79	NR			Kansas	NR
Gifford and Whitebread, 1951	T	Stomach only	4	NR			Pennsylvania	NR
Grimm and Roberts, 1950	T	Stomach only	18	NR			Pennsylvania	Fall-Winter
Hamilton, 1951	T & I	Stomach only	186	Yes (NA)			New York	May-Dec
Hopkins and Forbes, 1980	I	Stomach only	77	Yes (250 μm)			Oregon	Year round
Kasparian et al., 2002	T	Scat	75	Yes (600 μm)			Oklahoma	Year round
Knudsen and Hale, 1970	I	Stomach only	161	NA			Wisconsin	Year round
Lay, 1942	T	Stomach only	16	No			Texas	Sept
Llewellyn and Uhler, 1952	T	Digestive tract	37	No	Scat	66	Maryland	Year round
Reynolds, 1945	T	Stomach only	76	No	Scat	259	Missouri	Year round
Sandidge, 1953	T	Digestive tract	60	No			Kansas	Sept-Mar
Stieglitz and Klimstra, 1962	T	Digestive tract	131	NR			Illinois	Jan-Feb, Aug-Dec
Stuewer, 1943	T	Stomach only	15	NR	Scat	9	Michigan	NR
Sturgis, 1932	H & T	Digestive tract	30	NR	Scat	30	Michigan	July and Nov
Taube, 1947	T	Digestive tract	55	Yes (NA)			Michigan	Sept, Nov-Dec
Whitaker et al., 1977	H	Stomach only	83	NR			Indiana	NR
Wiseman and Hendrickson, 1950	T	Scat	87	No			Iowa	Jan-June
Wood, 1954	T	Digestive tract	25	Dried	Scat	23	Texas	Year round
Worth, 1975	I	Scat	NA	NR			New Jersey	Oct-Nov

larval stage of the 2-host ixodid tick, *Hyalomma marginatum*, required on average 9 days of feeding to achieve repletion. The Keesing et al. (2009) study maintained the opossums in captivity for four days after exposing them to larval ticks. It is possible, based on the literature regarding natural duration of feeding on hosts, that the opossums were not kept in captivity long enough for ticks to reach repletion and drop off. Temperature and restricted host movement could be contributing factors to this issue. If the lab was air-conditioned, the metabolism of the ticks may have slowed, lengthening the period to repletion (Ogden et al., 2004). Several studies show that ticks feed more slowly in cooler temperatures, lengthening the period that they will remain on the host (Sweatman, 1970; Norval, 1978; Lysyk, 2008). Opossums have a relatively slow metabolism and low body temperature compared to similarly sized placental mammals (McManus, 1974), which could contribute to a slower feeding rate and longer engorgement periods, especially in an air-conditioned lab. The researchers did not check the opossums for ticks before releasing them from captivity (F. Keesing, personal correspondence), having made the assumption that any tick still alive would have fed and dropped to the holding tray beneath the animals in the lab. Ergo, it is possible that ticks could have still been embedded and feeding on the opossums upon release. Furthermore, locomotive movement by the host is a stimulus for ticks to drop off of hosts after feeding (Apanaskevich and Oliver, 2014). Captive opossums in cages might not be engaging in ambulatory behaviors sufficient to stimulate repletion and subsequent drop off.

Extensive comparative research on animal behavior has identified myriad effects of captivity on stress responses (e.g., glucocorticoid levels, stereotypical behaviors, circadian shifts, aggressive grooming, fur-pulling behaviors, etc.) for many species of animals (McPhee and Carlstead, 2010). Other research has shown that bored animals may exhibit behaviors that do not align with their natural inclinations. Puhl et al. (2012) observed lab rats (*Rattus* spp.) self-administering cocaine more readily when they were in standard lab cages compared to rats in environmentally stimulating cages. As of yet, no research has compared the behaviors of individual opossums in their natural environments versus in captivity (but see McManus, 1970 and 1971, for captive opossum studies, including details regarding grooming behavior). If tick ingestion occurred due to fastidious grooming behavior, that behavior could have been stress-induced, as other studies of captive animal behavior suggest. It could also be an effect of boredom, as opossums in cages are restricted from normal activities such as foraging, intraspecific interactions, predator avoidance, and so forth. Otherwise we have to assume that opossums are naturally fastidious creatures, which doesn't logically align with the heavier burdens of ticks and fleas documented on live-trapped and hunted individuals. Another issue with the methods of Keesing et al. (2009) was the small sample size of captive opossums ($n = 5$), introducing the possibility of bias based on behavioral traits exhibited by a few individuals. Other researchers have noted that animals exhibit variation in behavior (Cavigelli, 2005), which may necessitate larger sample sizes to accurately capture the "normal" behavioral traits of a species.

The striking nondetection of ticks in multiple diet analyses, including ours, does not align with the claim of 5500 ticks being eaten per week, which would result in approximately 786 ticks being consumed per day during peak tick activity (5500/7 days). We acknowledge the difficulty in "proving a negative", but we strongly believe that, along with previous researchers, we would have found some evidence of ticks in stomachs if ticks were indeed a preferred diet item. Indeed, we would expect some ticks to be ingested even by accident if they are not sought after, but instead it appears that opossums may actively eschew the ingestion of ticks while grooming. Several specimens in our investigation had ticks embedded in the skin when we collected the stomachs, and yet those specimens had no ticks in the stomach contents. One opossum had 6 ticks embedded in the pinnae alone, which according to the calculations used in Keesing et al. (2009) would extrapolate to ~172 ingested ticks, and yet we found not even one tick or tick fragment in that animal's stomach. It appears that opossums are not seeking out ticks for consumption, even as part of the grooming process, and are possibly eschewing them. Another aspect of this dearth of ticks is perplexing: we expected that ticks would be ingested when other hosts were ingested. Further research may be required to explain this pronounced absence of ticks in opossum diets. Considering the small caloric payoff (approximately 3.37×10^{-4} J per tick, *sensu* Herrmann et al., 2013) for the investment required for digesting the chitinous exoskeleton of ixodid ticks, perhaps it should not be surprising that opossums do not focus their feeding efforts on ticks.

Our findings echo those of Şekercioğlu (2013), who investigated the stomach contents of 525 helmeted guinea fowl (*Numida meleagris)* and found only 5 ticks. Helmeted guinea fowl had been touted as a voracious tick consumer; consequently, humans introduced them to ecosystems all over the world as a biocontrol for ticks. Field studies (Ostfeld et al., 2006) indicated that helmeted guinea fowl were not voracious tick consumers, which Şekercioğlu (2013) confirmed with stomach contents analysis. Alarmingly, Şekercioğlu (2013) also found that helmeted guinea fowl introduced to Turkey likely served as nurseries for ticks; not the biocontrol they were hoped to be. As of yet, there are no official programs to introduce opossums as biocontrol agents. Given their carrier host habits, their synanthropic nature, their potential for long-distance dispersal, and their peripatric movement patterns (Hennessy et al., 2015), epidemiologists may want to consider monitoring opossum populations for tick-borne diseases.

This investigation was born from a discussion of a series of popular memes. The general public was enthused by the fantastic notion that the humble opossum was secretly solving the problem of tick-borne diseases by scarfing down ticks; this enthusiasm contributed to the wide broadcasting of this meme in many variations (Fig. 1). Furthermore, the belief that opossums voraciously consume ticks has influenced interpretation of interspecific animal behavior, as evinced by another viral meme that made the claim that the photograph shows an opossum benefitting a white-tailed deer (*Odocoileus virginianus*) by eating a tick off its face (Fig. 1b). There is no tick visible in the photo. The claim is unsupportable, yet contributes to a false understanding of ecosystem services and potentially obscures interpretation of the interspecific behavior. Viral claims that are later debunked undermine the public trust in experts and evidence-based science (Shiffman, 2019). Educating the public regarding complex zoonotic threats is challenging enough without misinformation muddying the waters (Polley, 2005). Our study demonstrates that scientists must be vigilant not only in regards to groundtruthing original research, but also in the peer-review process and the dissemination of those findings to the general public. Many comments in social media threads and in response to online articles demonstrate that members of the general public are interested in attracting opossums to their yards to serve as tick traps (Fig. 1f). Attracting wildlife to human residences increases the public's exposure to a wide range of zoonotic diseases and the public should be discouraged from engaging in this practice (Mackenstedt et al., 2015). This body of memes turned out to be an extremely successful advocacy campaign for the opossum; allowing the oft-maligned scavenger to achieve cult status as a biocontrol for ticks. Unfortunately, these purported benefits are not supported by our findings or by previous diet analyses. Our hope is that members of the public can still appreciate opossums for the roles they play in our ecosystems, even if that does not include eliminating ticks.

Declaration of Competing Interests

None.

Acknowledgements

We would like to thank Josh Wettstein, Ed Zimmerlein, and Karen Nemes for help with collecting opossum specimens. Kelly Fisher and Jen

Rocke were critical for library access and tracking down historical sources, especially during the COVID-19 shutdown. We are grateful to Katy Everett and Nicholas LaBonte for making aspects of this research possible. We are grateful to our supportive community at Eureka College for access to lab resources. We wish to thank managing editor, Olaf Kahl, and two anonymous but extremely helpful reviewers for their efforts in improving this manuscript.

Funding

This work was not funded by any funding source.

Ethics Statement

No animals were handled or euthanized for the purposes of this research. Scientific Permits for C. Hennessy from Illinois Department of Natural Resources include: NH17.6060, NH18.6060, and NH19.6060.

Data accessibility

All the data reported in this manuscript are the relevant data for this research.

Authors' contributions

C.H. collected and prepared specimens, investigated a smaller proportion of stomachs, and wrote the majority of the manuscript. K.H. investigated most of the stomachs and wrote an outline of the manuscript. Both authors collaborated on the identification of diet items.

References

Andrew, H.R., Norval, R.A.I., 1989. The role of males of the bont tick (*Amblyomma hebraeum*) in the transmission of *Cowdria ruminantium* (heartwater). Vet. Parasitol. 34, 15–23. https://doi.org/10.1016/0304-4017(89)90159-3.

Apanaskevich, D.A., Oliver, Jr.J.H., 2014. Life cycles and natural history of ticks. In: Sonnenshine, DE, Roe, RM (Eds.), Biology of Ticks. Oxford University Press, Oxford, UK, pp. 59–73.

Audubon, J.J., Bachman, J., 1851. The Viviparous Quadrupeds of North America, Vol. 2. V.G. Audubon, New York, New York, USA, pp. 107-125.

Barr, T.R., 1963. Infectious diseases in the opossum: a review. J. Wildl. Manag. 27, 53–71.

Bezerra-Santos, M.A., Ramos, R.A.N., Campos, A.K., Dantas-Torres, F., Otranto, D., 2021. *Didelphis* spp. opossums and their parasites in the Americas: a One Health perspective. Parasitol. Res. Pp 1–21. https://doi.org/10.1007/s00436-021-07072-4.

Black, J., 2020. Opossum removal best, as they carry ectoparasites. Pest Mgmt. Prof. https://www.mypmp.net/2020/05/06/opossum-removal-best-as-they-carry-ectoparasites/(accessed 15 Dec 2020).

Blumenthal, E.M., Kirkland Jr, G.L., 1976. The biology of the opossum, *Didelphis virginiana,* in southcentral Pennsylvania. Proc. Penns. Acad. Sci. 50, 81–85.

Brocke, H.R., 1970. The winter ecology and bioenergetics of the opossum, *Didelphis marsupialis*, as distributional factors in Michigan. Michigan State University, East Lansing, Michigan. Ph.D. dissertation.

Cavigelli, S.A., 2005. Animal personality and health. Behav 142, 1223–1244. https://doi.org/10.1163/156853905774539355.

Connolly, K., 2020. For better tick control, let nature lead the way. The Day, New London. Connecticut. https://www.theday.com/article/20200324/NWS01/200329810 (accessed 16 Dec 2020).

Cortinas, M.R., Kitron, U., 2006. County-level surveillance of white-tailed deer infestation by *Ixodes scapularis* and *Dermacentor albipictus* (Acari: Ixodidae) along the Illinois River. J. Med. Entomol. 43, 810–819. https://doi.org/10.1093/jmedent/43.5.810 https://digitalcommons.unl.edu/entomologyfacpub/237.

Dearborn, N., 1932. Foods of some predatory fur-bearing animals in Michigan. University of Michigan School of Forestry and Conservation Bulletin No. 1. University of Michigan Press, Ann Arbor, Michigan, USA.

Dexter, R.W., 1951. Earthworms in the winter diet of the opossum and raccoon. J. Mammal. 32, 464, 10.1093/jmammal/32.4.464a.

Durden, L.A., Richardson, D.J., 2013. Ectoparasites of the Virginia opossum (*Didelphis virginiana*), raccoon (*Procyon lotor*), and striped skunk (*Mephitis mephitis*) from Keith County. Nebraska. Trans. Neb. Acad. Sci. Affil. Soc. 420, 21–24.

Edmonds, P., 2019. If ticks bite, opossums bite back. Nat. Geo 236, 26.

Eisen, R.J., Eisen, L., Beard, C.B., 2016. County-scale distribution of *Ixodes scapularis* and *Ixodes pacificus* (Acari: Ixodidae) in the continental United States. J. Med. Entomol. 53, 349–386. https://doi.org/10.1093/jme/tjv237.

Feldner, M.K., 2019. The helpful opossum. https://vetmed.illinois.edu/wildlife/2019/06/05/the-helpful-opossum-2/(accessed 15 Dec 2020).

Fenger, C.K., Granstrom, D.E., Langemeier, J.L., Stamper, S., Donahue, J.M., Patterson, J. S., Gajadhar, A.A., Marteniuk, J.V., Xiaomin, Z., Dubey, J.P., 1995. Identification of opossums (*Didelphis virginiana*) as the putative definitive host of *Sarcocystis neurona*. J. Parasitol. 81, 916–919. https://doi.org/10.2307/3284040.

Fish, D., Daniels, T.J., 1990. The role of medium-sized mammals as reservoirs of *Borrelia burgdorferi* in southern New York. J. Wildl. Dis. 26, 339–345. https://doi.org/10.7589/0090-3558-26.3.339.

Fitch, H.S., Sandidge, L.L., 1953. Ecology of the opossum on a natural area in northeastern Kansas. Museum of Natural History Publication. University of Kansas, Lawrence, Kansas, USA, 7pp. 305-338.

Gardner, A.L., 1982. Virginia opossum. In: Chapman, J., Feldhamer, G. (Eds.), Wild Mammals of North America: Biology, Management, and Economics. John Hopkins University Press, Baltimore, Maryland, USA, pp. 3–36.

Gifford, C.L., Whitebread, R., 1951. Mammal Survey of South Central Pennsylvania: Final Report, Pittman-Robertson Project 38-R. Pennsylvania Game Commission, Harrisburg, Pennsylvania, USA, pp. 1–75.

Grimm, W.C., Roberts, H.A., 1950. Mammal Survey of Southwestern Pennsylvania. Pennsylvania Game Commission, Harrisburg, Pennsylvania, USA, 1-99.

Hamilton Jr., W.J., 1951. The food of the opossum in New York State. J. Wildl. Manage. 15, 258–264. https://doi.org/10.2307/3797218.

Hamilton Jr., W.J., 1958. Life history and economic relations of the opossum (*Didelphis marsupialis virginiana*) in New York State. Cornell Univ. Agric. Exp. Sta. Mem. 354, 1–48, 354, 1-48.

Hanganu-Bresch, C., 2017. What's in a meme? In: Ratliff, C. (Ed.), The 2016 CCCC Intellectual Property Annual. Top Intellectual Property Developments of 2016. https://escholarship.org/uc/item/2fp6t2g3 (Accessed 15 Dec 2020).

Hennessy, C., Tsai, C.C., Beasley, J.C., Beatty, W.S., Zollner, P.A., Rhodes Jr., O.E., 2015. Elucidation of population connectivity in synanthropic mesopredators: using genes to define relevant spatial scales for management of raccoons and Virginia opossums. J. Wildl. Manage. 79, 112–121. https://doi.org/10.1002/jwmg.812.

Herrmann, C., Voordouw, M.J., Gern, L., 2013. *Ixodes ricinus* ticks infected with the causative agent of Lyme disease, *Borrelia burgdorferi* sensu lato, have higher energy reserves. Int. J. Parasitol. 43, 477–483. https://doi.org/10.1016/j.ijpara.2012.12.010.

Hopkins, D.D., Forbes, R.B., 1980. Dietary patterns of the Virginia opossum in an urban environment. Murrelet 61, 20–30 https://doi:10.2307/3536187.

Illinois Department of Public Health, 2019. Common Ticks. https://www.dph.illinois.gov/sites/default/files/publications/2019-tickborne-disease-exposure-mapnovember.pdf (accessed 4 June 2021).

Kasparian, M.A., Hellgren, E.C., Ginger, S.M., 2002. Food habits of the Virginia opossum during raccoon removal in the Cross Timbers Ecoregion. Oklahoma. Proc. Ok. Acad. Sci. 82, 73–78.

Keesing, F., Brunner, J., Duerr, S., Killilea, M., LoGiudice, K., Schmidt, K., Vuong, H., Ostfeld, R.S., 2009. Hosts as ecological traps for the vector of Lyme disease. Proc. R. Soc. B 276, 3911–3919. https://doi.org/10.1098/rspb.2009.1159.

Kitron, U., Jones, C.J., Bousemen, J.K., 1991. Spatial and temporal dispersion of immature *Ixodes dammini* on *Peromyscus leucopus* in northwestern Illinois. J. Parasitol. 77, 945–949. https://doi.org/10.2307/3282747.

Knight, M.M., Norval, R.A.I., Rechav, Y., 1978. The life cycle of the tick *Hyalomma marginatum rufipes* Koch (Acarina: Ixodidae) under laboratory conditions. J. Parasitol 64, 143–146. https://doi.org/10.2307/3279627.

Knudsen, G.J., Hale, J.B., 1970. Food habits of opossums in southern Wisconsin. Wisconsin Dept. of Nat. Resources Report 61, 1–11.

Kollars Jr., T.M., 1993. Ticks (Acari: Ixodidae) infesting medium-sized wild mammals in southwestern Tennessee. J. Med. Entomol. 30 https://doi.org/10.1093/jmedent/30.5.896, 896-90.

Krueger, L., Bai, Y., Bennett, S., Fogarty, C., Sun, S., Kosoy, M., Maina, A., Nelson, K., Platzer, E., Osikowicz, L., Richards, A.L., Shariar, F., Trinidad, A., Cummings, R., 2016. Identification of zoonotic and vector-borne infectious agents associated with opossums (*Didelphis virginiana*) in residential neighborhoods of Orange County. California. Proc. Vert. Pest Conf. 27, 268–279. https://doi.org/10.5070/V427110386.

Lay, D.W., 1942. Ecology of the opossum in eastern Texas. J. Mammal. 23, 147–159 https://doi: 10.2307/1375067.

Levi, T., Keesing, F., Holt, R.D., Barfield, M., Ostfeld, R.S., 2016. Quantifying dilution and amplification in a community of hosts for tick-borne pathogens. Ecol. App. 26, 484–498. https://doi.org/10.1890/15-0122.

Lipske, M., 2015. Give opossums a break. National Wildlife Foundation https://www.nwf.org/Magazines/National-Wildlife/2015/AprilMay/Animals/Opossums (accessed 15 Dec 2020).

Llewellyn, L.M., Uhler, F.M., 1952. The foods of fur animals of the Patuxent Research Refuge. Maryland. Am. Midl. Nat. 48, 193–203 https://doi:10.2307/2422142.

Lockhart, J.M., Davidson, W.R., Stallknecht, D.E., Dawson, J.E., Howerth, E.W., 1997. Isolation of *Ehrlichia chaffeensis* from wild white-tailed deer (*Odocoileus virginianus*) confirms their role as natural reservoir hosts. J. Clin. Microbio. 35, 1681–1686. https://doi.org/10.1146/annurev.ento.48.091801.112728.

Lysyk, T.J., 2008. Effects of ambient temperature and cattle skin temperature on engorgement of *Dermacentor andersoni*. J. Med. Entomol. 45, 1000–1006. https://doi.org/10.1093/jmedent/45.6.1000.

Mackenstedt, U., Jenkins, D., Romig, T., 2015. The role of wildlife in the transmission of parasitic zoonoses in peri-urban and urban areas. Int. J. Parasitol. Parasites Wildl. 4, 71–79. https://doi.org/10.1016/j.ijppaw.2015.01.006.

Marshall, S.A., 2006. Insects: Their Natural History and Diversity. *Insects: Their Natural History and Diversity*. Firefly Books, Richmond Hill.

Martins, T.F., Moura, M.M., Labruna, M.B., 2012. Life-cycle and host preference of *Amblyomma ovale* (Acari: ixodidae) under laboratory conditions. Exp. App. Acarol. 56, 151–158 https://doi.org/10.1007/s10493-011-9506-9.

McManus, J.J., 1970. Behavior of captive opossums, *Didelphis marsupialis virginiana*. Am. Midl. Nat. 84, 144–169 https://doi.org/10.2307/2423733.

McManus, J.J., 1971. Activity of captive *Didelphis marsupialis*. J. Mammal. 52, 846–848 https://doi.org/0.2307/1378945.

McManus, J.J., 1974. *Didelphis virginiana*. Mammalian Species 40, 1–6 https://doi.org/10.2307/3503783.

McPhee, M.E., Carlstead, K., 2010. The importance of maintaining natural behaviors in captive mammals. In: Kleiman, D.G., Thompson, K.V., Baer, C.K. (Eds.), Wild Mammals in Captivity: Principles and Techniques for Zoo Management. University of Chicago Press, Chicago, Illinois, USA, pp. 303–313. Second Edition.

Miller, R., 2014. Opossums - killers of ticks. Newstimes https://www.newstimes.com/news/article/Robert-Miller-Opossums-killers-of-ticks-5413872.php.

Norval, R.A.I., 1978. Repeated feeding of *Amblyomma hebraeum* (Acarina: Ixodidae) immatures on laboratory hosts. Host effects on tick yield, engorged weight and engorgement period. J. Parasitol 64, 910–917. https://doi.org/10.2307/3279530.

Ogden, N.H., Lindsay, L.R., Beauchamp, G., Charron, D., Maarouf, A., O'Callaghan, C.J., Waltner-Toews, D., Barker, I.K., 2004. Investigation of relationships between temperature and developmental rates of tick *Ixodes scapularis* (Acari: Ixodidae) in the laboratory and field. J. Med. Entomol 41, 622–633. https://doi.org/10.1603/0022-2585-41.4.622.

Ostfeld, R.S., Price, A., Hornbostel, V.L., Benjamin, M.A., Keesing, F., 2006. Controlling ticks and tick-borne zoonoses with biological and chemical agents. Biosci 56, 383–394.

Polley, L., 2005. Navigating parasite webs and parasite flow: emerging and re-emerging parasitic zoonoses of wildlife origin. Intl. J. Parasitol. 35, 1279–1294. https://doi.org/10.1016/j.ijpara.2005.07.003.

Puhl, M.D., Blum, J.S., Acosta-Torres, S., Grigson, P.S., 2012. Environmental enrichment protects against the acquisition of cocaine self-administration in adult male rats, but does not eliminate avoidance of a drug-associated saccharin cue. Behav. Pharm. 23, 43–53. https://doi.org/10.1097/FBP.0b013e32834eb060.

Reynolds, H.C., 1945. Some aspects of the life history and ecology of the opossum in central Missouri. J. Mammal. 26, 361–379.

Sandidge, L.L., 1953. Food and dens of the opossum (*Didelphis virginiana*) in northeastern Kansas. Trans. Kan. Acad. Sci. 56, 97–106.

Schumacher, L., Snellgrove, A., Levin, M.L., 2016. Effect of *Rickettsia rickettsii* (Rickettsiales: Rickettsiaceae) infection on the biological parameters and survival of its tick vector—*Dermacentor variabilis* (Acari: Ixodidae). J. Med. Entomol. 53, 172–176. https://doi.org/10.1093/jme/tjv166.

Şekercioğlu, Ç.H., 2013. Guineafowl, ticks and Crimean–Congo hemorrhagic fever in Turkey: the perfect storm? Trends Parasitol 29, 1–2. https://doi.org/10.1016/j.pt.2012.10.001.

Shiffman, D.S., 2019. The danger of viral falsehoods in conservation. Macroscope Blog https://www.americanscientist.org/blog/macroscope/the-danger-of-viral-falsehoods-in-conservation (accessed 16 Dec 2020).

Stieglitz, W.O., Klimstra, W.D., 1962. Dietary pattern of the Virginia opossum, *Didelphis virginianus* Kerr, late summer-winter, southern Illinois. Trans. Ill. Acad. Sci. 55, 198–208.

Stuewer, F.W., 1943. Raccoons: their habits and management in Michigan. Ecol. Monographs 13, 203–257. https://doi.org/10.2307/1943528.

Sturgis, I.S.J., 1932. A Comparative Study of the Food Habits of the Skunk and Opossum in Michigan. A comparative study of the food habits of the skunk and opossum in Michigan. University of Michigan, Ann Arbor. Masters thesis.

Sweatman, G.K., 1970. Temperature control of engorgement by subadult *Hyalomma aegyptium* ticks. J. Med. Entomol. 7, 71–78. https://doi.org/10.1093/jmedent/7.1.71.

Taube, C.M., 1947. Food habits of Michigan opossums. J. Wildl. Manage. 11, 97–103. https://doi.org/10.2307/3796046.

Taylor, L.H., Latham, S.M., Woolhouse, M.E., 2001. Risk factors for human disease emergence. Phil. Trans. R. Soc. B 356, 983–989. https://doi.org/10.1098/rstb.2001.0888.

Tuten, H.C., Burkhalter, K.L., Noel, K.R., Hernandez, E.J., Yates, S., Wojnowski, K., Hartleb, J., Debosik, S., Holmes, A., Stone, C.M., 2020. Heartland virus in humans and ticks, Illinois, USA, 2018–2019. Emerg. Infect. Dis. 26 (7), 1548–1552. https://doi.org/10.3201/eid2607.200110.

Walsh, L.L., Tucker, P.K., 2018. Contemporary range expansion of the Virginia opossum (*Didelphis virginiana*) impacted by humans and snow cover. Can. J. Zool. 96, 107–115. https://doi.org/10.1139/cjz-2017-0071.

Wenzel, R.L., Tipton, V.J., 1966. Some relationships between mammal hosts and their ectoparasites. In: Wenzel, R.L., V.J. Tipton (Eds.), Ectoparasites of Panama. Field Museum of Natural History, Chicago, IL, USA, pp. 677-723.

Whitaker Jr., J.O., Jones, G.S., Goff, R.J., 1977. Ectoparasites and food habits of the opossum, *Didelphis virginiana*, in Indiana. Proc. Ind. Acad. Sci. 86, 501–507.

Wiseman, G.L., Hendrickson, G.O., 1950. Notes on the life history and ecology of the opossum in southeast Iowa. J. Mammal. 31, 331–337. https://doi.org/10.2307/1375304.

Wood, J.E., 1954. Food habits of furbearers of the upland post oak region in Texas. J. Mammal. 35, 406–415. https://doi.org/10.2307/1375965.

Worth, C.B., 1975. Virginia opossums (*Didelphis virginiana*) as disseminators of the common persimmon (*Diospyros virginiana*). J. Mammal. 56 https://doi.org/10.2307/1379381, 517-517.

Yabsley, M.J., Shock, B.C., 2013. Natural history of zoonotic Babesia: role of wildlife reservoirs. Intl. J. Parasitol. Parasites Wildl. 2, 18–31. https://doi.org/10.1016/j.ijppaw.2012.11.003.

Willson, J., July 17, 2017. How to Attract a Possum to My Yard. https://www.hunker.com/13425595/how-to-attract-a-possum-to-my-yard (accessed 26 Mar 2021).

Hetzler, P., March 28, 2020. More about opossums. The Adirondack Almanack. https://www.adirondackalmanack.com/2020/03/more-about-opposums.html (accessed 15 Dec 2020).