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Parasites and vector-borne pathogens of southern plains woodrats (*Neotoma micropus*) from southern Texas

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Abstract

From 2008–2010, southern plains woodrats (*Neotoma micropus*) from southern Texas, were examined for parasites and selected pathogens. Eight helminth species were recovered from 97 woodrats including, *Trichuris neotomae* from 78 (prevalence=80%), *Ascarops* sp. from 42 (43%), *Nematodirus neotoma* from 31 (32%), *Raillietina* sp. from nine (9%), *Taenia taeniaeformis* larvae from eight (8%), and an unidentified sp. from a single woodrat. *Besnotia neotomofelis* was detected in three (3%) woodrats and microfilaria were detected in seven (7%). PCR testing of blood samples from 104 woodrats detected a novel *Babesia* sp. in one (1%) and *Hepatozoon* sp. in 17 (16%) woodrats. Partial 18S rRNA gene sequence of the *Babesia* was 94% similar to *B. conradae*. Histologic examination of tissues detected intestinal coccidia in 7 of 104 (7%), *Sarcocystis neotomafelis* in 26 (25%), *Hepatozoon* sp. in 21 (20%), and *Dunnifilaria meningica* in four (4%) woodrats. Three woodrats (5%) were seropositive for *Toxoplasma gondii*. Ectoparasites recovered included fleas (*Orchopeas sexdentatus* and *O. neotomae*), ticks (*Ixodes woodi* and *Ornithodoros turicata*), mites (*Trombicula* sp. and *Ornithonyssus (Bdellonyssus) bacoti*) and bot flies (*Cuterebra* sp.). The only difference in prevalence related to gender was for *N. neotoma* (males > females, $p=0.029$). Prevalence of *T. neotomae* and all intestinal parasites combined was significantly higher in adults compared with juveniles ($p=0.0068$ and $p=0.0004$), respectively. Lesions or clinical signs were associated with *Cuterebra*, *T. gondii*, and *B. neotomofelis*. Collectively, these data indicate that woodrats from southern Texas harbor several parasites of veterinary and/or medical importance.

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Conflict of interest statement

No competing financial interests exist for any author.

Keywords

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Introduction

The southern plains woodrat (*Neotoma micropus*), commonly called a packrat, is a medium-sized, nocturnal rodent that inhabits semi-arid brush lands, low valleys and plains of the south-central and southwestern United States and northeastern Mexico. In Texas, *N. micropus* inhabits areas dominated by thorny desert shrubs or cacti (Braun and Mares 1989) and their diet consists mainly of vegetation such as succulent leaves and fruit of cacti, seeds and acorns (Raun 1966). Woodrats (*Neotoma* spp.) are common hosts for ticks and fleas which are potential vectors of tularemia (*Francisella tularensis*), plague (*Yersinia pestis*), Q fever (*Coxiella burnetti*), relapsing fever (*Borrelia* spp.) and Rocky Mountain spotted fever (*Rickettsia rickettsi*). Other pathogenic organisms reported from woodrats include *Trypanosoma cruzi* (causative agent of Chagas' disease in humans and domestic animals), *Besnoitia neotomofelis*, and *Leishmania mexicana* (McHugh et al. 1990; Dubey and Yabsley 2010; Pinto et al. 2010).

Although numerous studies have looked at the ectoparasitic fauna of woodrats in Texas, to date, only a few studies have looked at endoparasites of southern plains woodrats. Collectively, in the United States and Mexico, only nine species have been reported including: *Taenia taeniaeformis*, *Litomosoides carinii*, *Dunnifilaria meningica*, *Trichuris muris*, *L. mexicana*, *Try. cruzi*, *Try. neotomae*, *Sarcocystis neotomafelis*, and *B. neotomofelis* (Packchianian 1942; Johnson 1966; Burkholder et al. 1980; Gutierrez-Pena 1989; Galaviz-Silva et al. 1991; Pinto et al. 2010; Charles et al. 2011). Because higher diversities of parasites have been reported in other species of woodrats in the southwestern United States, we conducted this study to better understand the endo- and ectoparasitic fauna of southern plains woodrats from Uvalde County, Texas.

Materials and Methods

Trapping

A total of 104 southern plains woodrats (56 females and 48 males) were trapped during July 2008 and March and May 2010 at four sites in Uvalde County, Texas. Animals were live trapped by small squirrel cage traps (Havahart, Litz, Pennsylvania) and large Sherman traps (H.B. Sherman Traps, Tallahassee, Florida) baited with dried apricots. Trap stations were selected based on fresh tracks and rodent droppings at the base of presumed woodrat nests built among cactus (*Opuntia* spp.) plants. Traps were set in the afternoon and checked the following morning.

Anesthesia and blood collection

Captured animals were anesthetized and weighed. Briefly, woodrats were anesthetized with 100mg/kg ketamine (Fort Dodge Laboratories, Fort Dodge, Iowa) followed by blood collection via cardiocentesis into potassium ethylenediaminetetraacetic acid (K₂EDTA) BD Vacutainer® tubes (Beckton Dickinson, Franklin Lakes, New Jersey) using aseptic techniques. In 2010, blood smears were made with fresh blood, air-dried, fixed in absolute alcohol for five minutes, and stained with Geimsa stain. All animals were euthanized by cervical dislocation and adult and juvenile (not pups) were then necropsied and examined for parasites. All techniques were reviewed and approved by the IACUC committee at the University of Georgia.

Parasite collection and identification

Each woodrat was examined for ectoparasites by combing back the fur and collecting specimens with fine forceps. Collected ectoparasites were preserved in 100% ethanol. Bot-fly larvae were removed by gentle traction with forceps and characterized using polymerase chain reaction (PCR) and sequencing of two regions of the cytochrome oxidase subunit I (COI) gene as described in Table 1. Fleas, mites, and ticks were mounted on slides using saline solution and identified to species with a light microscope and published taxonomic keys (Eads 1950; Keirans and Litwak 1989; Lewis 2000; Haas et al. 2004).

During necropsy, the viscera of all woodrats were grossly examined for the presence of parasites such as *Taenia* and *Besnoitia* cysts. The entire length of gastrointestinal tract and some organs (pancreas, liver, and spleen) were removed from the abdominal cavity, dissected under a dissecting scope, and closely examined for helminths. Contents were filtered through a 100µm sieve (W.S. Tyler Incorporated, Mentor, Ohio) for concentration of parasites. All parasites were stored in 100% ethanol and examined under a light or dissecting microscope for identification. Large nematodes were cleared with a 70% ethanol/30% phenol solution.

Histopathology

Tissue samples including brain, lung, liver, heart, kidney, spleen, lymph nodes, quadriceps, gonads and sections of the gastrointestinal tract were preserved in 10% buffered formalin for histopathological examination. Small sections of formalin-fixed tissues were embedded in paraffin, sectioned at 5µm and stained with hematoxylin and eosin (H&E) for light microscopic examination.

Serologic testing for *Toxoplasma gondii*

Sera samples from 66 woodrats were tested for antibodies to *T. gondii* by the modified agglutination test (MAT) as described by Dubey and Desmonts (1987).

Molecular detection of parasites

Several PCR assays were used to test woodrats for hemoparasites and vector-borne bacteria. DNA was extracted from 100µL of whole blood using the DNeasy blood and tissue kit (Qiagen, Inc., Valencia, CA) according to the manufacturer's protocol. The extracted DNA was used as a template to test for species of *Babesia*, *Hepatozoon*, and *Rickettsia* as described in Table 1. Briefly, for primary amplification, 5µL of DNA was added to 20µL of a master mix containing 11µL of molecular grade biological water (MGBW), 2.5µL of 25mM MgCl₂, 5µL of GoTaq Flexi Clear Buffer (Promega, Madison, Wisconsin), 0.25µL of 20mM dNTP, 0.5µL of each primer (40µM), 0.25µL of GoTaq Flexi (Promega). For each secondary reaction (if needed), 1µL of primary product was used as a template in a 25µL reaction containing similar PCR components with the exception of an additional 4µL of water and different primers.

Stringent protocols and controls were utilized in all PCR reactions to prevent and detect contamination. Separate dedicated laboratory areas were used for DNA extraction, primary amplification, secondary amplification, and product analysis. A negative water control was included in each set of DNA extractions, and one water control for each set of primary and secondary PCR reactions. Amplicons were visualized by trans-illumination of an ethidium bromide-stained 1.5% agarose gel.

Statistical analyses

Parasite prevalence, intensity and range were calculated as defined by Bush et al. (1997). Fisher's exact test was used to test for differences in parasite prevalence (by species and collectively) among age classes and gender. A two-way ANOVA implemented by SAS, was used to determine if gastro-intestinal nematode intensity varied according to age and/or gender.

Results

A total of nine helminth species were recovered from 90 of 97 woodrats (54 females/43 males and 79 adults/18 juveniles) including four species of nematodes (*Trichuris neotomae*, *Nematodirus neotoma*, *Ascarops* sp. and a single unidentified female spuriid), two species of cestodes (*Raillietina* sp. and *Taenia taeniaeformis*) and two species of trematodes (*Scaphiostomum* sp., a pancreatic fluke, and *Zonorchis* sp., a liver fluke) (Table 2). Additionally, microfilaria of an unknown filarial nematode species (likely either *Litosomoides carinii* or *Dunnifilaria meningica*) was found in the blood. The *T. taeniaeformis* cysticerci were found encysted in the liver and the adult stage of a *Raillietina* sp. was found in the lumen of the small intestine. The three nematodes were all found in the gastrointestinal tract, *Ascarops* sp. in the stomach, *N. neotomae* in the small intestine, and *T. neotomae* in the large intestine (Table 2). The only helminth that was associated with gross lesions was the *Ascarops* sp. which were surrounded by areas of thickened gastric mucosa (~1cm diameter) which housed multiple worms, up to 17 in one case. Seven woodrats (three females/four males and two adults/five juveniles) were negative for all helminths.

No difference in prevalence of whipworms was noted for gender ($p=1.000$) but adults had a significantly higher prevalence compared with juveniles ($p=0.0068$). In contrast, prevalence of *N. neotomae* was significantly higher in males (19 of 43) compared to females (12 of 54) ($p=0.0285$), but no difference was noted for age ($p=0.165$). Overall gender was not associated with differences in prevalence of intestinal parasites ($p=0.460$) but age was, with more adults 77 of 79 (98%) being infested than juveniles (12 of 18 (67%) ($p=0.0004$)). Based on ANOVA, the interaction of age and gender on parasite intensity for all gastro-intestinal nematodes was not significant ($p>0.05$).

A total of 181 ectoparasites (118 fleas, 9 ticks, 39 mites and 15 bots) were collected from 42 of the 104 (40%) woodrats (Table 3). Bot fly larvae were found predominantly on the chest and neck regions of infested woodrats but one (first instar) larva was found in the nasal cavity. A single woodrat (juvenile female, weight, 118g) that had 4 larvae (1.5–2 cm in length) under the chin and on the chest region was found severely emaciated, lethargic, and non-responsive a few inches outside one of our traps. We were able to collect the woodrat by hand after which it died while we performed an external examination. Sequence of a fragment of the COI gene (411 bp) from a single bot fly was 87.9% similar to *C. grisea* (AY507182) and 86.8% similar to *C. fontinella* (AY507188). Sequences of another region of the COI gene (544bp) from two bot fly larvae from two different animals were identical and 90.4% similar to *C. fontinella* (AY507197) and 89.9% similar to *C. grisea* (AY507222).

Based on PCR testing and sequencing, a novel *Babesia* was detected in 1 of 42 (2%) woodrats in 2008; all were negative in 2010 (Table 4). Partial 18S rRNA gene sequence of the *Babesia* species was most similar (434 of 460bp, 94.4% similarity) to *B. conradae* (AF158702), a parasite of domestic dogs in California. Other similar *Babesia* species included *B. lengau* (94.1%) from cheetahs (*Acinonyx jubatus*) (GQ411405-GQ411417), *B. vesperuginis* (92.4%) from a *Pipistrellus* sp. bat from England (AJ871610), and *B. duncani* (92%) from humans in California (AY027815). *Hepatozoon* sp. was commonly detected in blood samples of woodrats by PCR (Table 4); no gamonts were observed in blood smears.

All woodrat blood samples were PCR negative for *Rickettsia*. Three of 66 (5%) woodrats were positive for antibodies to *T. gondii* (titers of 1:25, 1:25, and 1:400). Additionally, *T. gondii* was isolated from digested brain, heart and tongue tissue from one seronegative woodrat, but not from any of the three seropositive woodrats (data not shown, Dubey et al., 2011).

Several parasites were detected during histologic examination of tissues. A meningeal worm (*Dunnifilaria meningica*), associated with mild lymphocytic and eosinophilic meningitis, was observed in the meningeal and sub-meningeal spaces of four (4%) woodrats. Sarcocysts of *S. neotomafelis* were observed in 26 of 104 (25%) woodrats with more sarcocysts being found in the quadriceps muscle (26/26) (100%) compared with the myocardium (4/26) (15%). *S. neotomafelis* cysts ranged from a few (3–4) to numerous (>50) per tissue sample. A few cysts were accompanied with a mild multifocal myositis. *Hepatozoon* cysts were observed in the liver of 21 (20%) woodrats compared to 17 (16%) that were positive with PCR of blood samples. Only six woodrats were positive for *Hepatozoon* sp. by PCR and histology. Two encysted flukes surrounded by fibrous capsules were found in the liver of one woodrat. Based on histology, *Besnoitia neotomofelis* was detected in the tissues of two woodrats (2%). A third woodrat, which was moribund at capture, had numerous grossly visible cysts present in the facial skin and throughout the musculature and subcutaneous layer of the body (Charles et al. 2011). Developmental stages (micro- and macrogametocytes and oocysts) of coccidia were found in the small and large intestine of seven (7%) woodrats.

Discussion

Southern plains woodrats are known to be hosts for a suite of parasites, some of which are of medical and veterinary importance (Packchanian 1942; Johnson 1966). Considerable work has been done to characterize the ectoparasitic fauna of southern plains woodrats, but to date, there is a paucity of reported information on the diversity and prevalence of parasites and vector-borne pathogens in this rodent species. In this current study, we report eight new parasite-host associations including one flea (*Orchopeas neotomae*), two trematodes (*Scaphiostomum* sp. and *Zonorchis* sp.), one stomach nematode (*Ascarops* sp.), an intestinal nematode (*N. neotoma*), and three protozoan parasites (*T. gondii*, *Babesia* sp., and coccidia). Additionally, we report *S. neotomafelis* infection in *N. micropus* for the first time in the US. Collectively, these data indicate that southern plains woodrats in Uvalde Co., Texas are hosts to several parasites and pathogens, and although most were not associated with significant gross or histological lesions, some may cause disease in humans, woodrats, and other animals.

All ectoparasites recovered from the woodrats in this study have been previously recorded from this host with the exception of *O. neotomae* (Johnson 1966). This flea species feeds primarily on *Neotoma* spp., especially the Mexican woodrat (*N. mexicana*). The range of *N. mexicana* overlaps with that of *N. micropus* in New Mexico, far western Texas, and parts of Mexico but not in our study area. Similar to our findings, Stark (1958) found co-infestations of with *O. neotomae* and *O. sexdentatus* on the same host (Stark 1958). Both flea species are involved in the host-flea complex in the spread of *Yersinia pestis* in western United States (Anderson and Williams 1997).

Flies of the genus *Cuterebra* are very common in most temperate and tropical regions in the western hemisphere (Sabrosky 1986). Larvae of these flies infest the subcutis of lagomorphs and rodents (including *Neotoma* spp.). Although bots are relatively large compared to their hosts, they rarely cause mortality. Young animals are more prone to injury or increased susceptibility to predation if larval burdens are high. In this study two infested woodrats

exhibited good body condition, but one juvenile woodrat that was infested with four bots ~2cm long, had poor body condition, and was likely moribund due to *Cuterebra* infestation. Sequences from our woodrat samples were most similar to *C. fontinella* and *C. grisea* (only two *Cuterebra* spp. in Genbank) which are both parasites of mice in the genus *Peromyscus* (Noel et al. 2004). Bot flies are common in woodrats, but unfortunately there are no sequences available for the many *Cuterebra* spp. reported from *Neotoma* spp. for comparison with the sequences we obtained (Baird 1979; Baird 1997; Wilson et al. 1997).

Several of the helminths detected in this study are common parasites of *Neotoma* spp. including *Trichuris* spp., *N. neotomae*, *Raillietina* sp., and *T. taeniaeformis*. Prevalence of *Trichuris* spp. in woodrats is typically very high; a previous study of *N. micropus* reported *T. muris* in all four woodrats examined (Johnson 1966). *T. muris* (likely now considered to be *T. neotomae*) has also been reported from the eastern woodrat (*N. floridana*) from Oklahoma and *T. neotomae* has been reported from the dusky-footed woodrat (*N. fuscipes*) in California (Chandler 1945; Boren et al. 1993). Although this is the first report of *N. neotoma* (= *N. tortuosus*) (Hoberg et al. 1988) in *N. micropus*, it has been reported from numerous other woodrat species including *N. fuscipes*, the bushy-tailed woodrat (*N. cinerea*), the desert woodrat (*N. lepida*), *N. mexicana*, and *N. floridana* (Hall 1916; Tucker 1942; Miller and Schmidt 1982). Neither of these helminthes has been associated with disease in woodrats.

Woodrats were hosts to two different species of tapeworms, *T. taeniaeformis* which were found as larvae in the liver and a *Raillietina* sp. that was found as adults in the small intestine. *T. taeniaeformis* has been reported from southern plains woodrats previously with 26% of 88 being positive (Johnson 1966). Strobilocerci of *T. taeniaeformis* are commonly found in the liver of woodrats, rabbits, squirrels, muskrats, bats, voles, other small rodents and occasionally humans and the adult form in felids which are definitive hosts (Johnson 1966; Theis and Schwab 1992; Fichet-Calvet, et al. 2003). Rarely does this parasite cause any significant lesions or disease in intermediate hosts; however, high numbers can cause infertility and hepatic neoplasia in some rodents (Lin et al. 1990; Irizarry-Rovira et al. 2007). Although *Raillietina* spp. are uncommon in woodrats, they have been reported in *N. cinerea*, *N. fuscipes*, and *N. lepida* (Linsdale and Tevis, 1951; Grundmann 1958; Miller and Schmidt 1982).

Prior to this study, no trematode species have been reported from *Neotoma* spp. We detected at least two species, *Scaphiostomum* sp. and *Zonorchis* sp., and a possible third species was found encysted in the liver of one woodrat that is not believed to be *Zonorchis* (typically found in the gall bladder, bile ducts or small intestine proximal to the bile duct opening) (McIntosh 1939; Santos et al. 2010). *Scaphiostomum* spp. are usually found in the pancreatic duct of its definitive rodent hosts, including the white-ankled mouse (*Peromyscus pectoralis*) in Texas (Santos et al. 2010). The primary intermediate host is the terrestrial flamed disc snail, *Anguispira alternata*, and secondary intermediate terrestrial snail hosts include *Neohelix* (= *Triodopsis*) *albolaris* and *Haplotrema concavum* (Schell 1985). *Zonorchis* sp. has also been reported from the white-ankled mice in Texas, but this parasite utilizes the terrestrial snail *Polygyra texasiana* as an intermediate host (Schell 1985). The low prevalence of *Scaphiostomum* sp. and *Zonorchis* sp. is likely due to a low density of appropriate intermediate hosts or because these snails are rarely consumed by *N. micropus*.

A novel *Babesia* sp. was detected in a single woodrat from our 2008 collection. Unfortunately, no blood smears were made in 2008 so no morphologic data is available. Based on partial 18S rRNA gene sequence, this woodrat *Babesia* was most similar to *B. conradae*, a canine species from California (Kjemtrup et al. 2006). This represents the first report of a *Babesia* species in a *Neotoma* spp.; however, a different piroplasm, *Theileria*

youngi, has been reported from *N. fuscipes* from California (Kjemtrup et al. 2001). Possible vectors include *I. woodi*, a common tick found in the current study and previous studies (Eads et al. 1952; Johnson 1966) and the less commonly found ticks, *Amblyomma inornatum*, *Dermacentor variabilis*, and *D. parumapertus* (Eads et al. 1952; Eads and Hightower 1956; Johnson 1966).

Based on histology and PCR testing of blood, *Hepatozoon* infections were common in this woodrat species. Recently, genetic characterization of *Hepatozoon* sp. from southern plains woodrats found it was related to a *Hepatozoon* sp. from *N. fuscipes* in California as well as other rodent *Hepatozoon* (Allen et al. 2011). Similar to a study of *Hepatozoon* in cotton rats (*Sigmodon hispidus*), meronts were present in the liver of our naturally infected woodrats (Johnson et al. 2007). Interestingly, *Hepatozoon* from *N. fuscipes* and *N. micropus* were genetically similar to *Hepatozoon* detected in snakes and other rodents (Allen et al. 2011). Genetic characterization of *Hepatozoon* from snakes, as potential hosts, in southern Texas may clarify the natural history of this understudied group of parasites.

Three tissue cyst-forming coccidians, *S. neotomafelis*, *B. neotomofelis*, and *T. gondii*, were detected in woodrats in the current study and interestingly, all are suspected to utilize felids as definitive hosts. Although *S. neotomafelis* was common in the woodrats in the current study, this parasite had previously only been reported in *N. micropus* from Nuevo Leon, Mexico (Galaviz-Silva et al. 1991). Only sections of heart and quadriceps were examined in this present study and cysts were more prevalent in quadriceps muscles whereas in a previous study, cysts were more common in the masseter muscles and only rarely found in quadriceps muscles (Galaviz et al., 1991). Currently, the only suspected definitive host for *S. neotomafelis* is the domestic cat; however, the only study that has attempted to experimentally determine the life cycle depicts an oocyst passed in cat feces that is morphologically more similar to *Isospora* than *Sarcocystis* (Galaviz-Silva et al. 1991). However, we believe that felids could be the definitive host because a recent study found a *Sarcocystis*-infected woodrat co-infected with two other felid-transmitted protozoans, *Besnoitia neotomofelis* and *T. gondii* (Charles et al. 2011). Cysts of *B. neotomofelis* were detected in low numbers in two woodrats which had no lesions associated with infection; however, the finding of a single woodrat that was moribund due to *Besnoitia* infection indicates that *B. neotomofelis* can be a cause of clinical disease (Charles et al., 2011). Although *Besnoitia* spp. are typically considered nonpathogenic, *B. jellisoni*, a parasite of deer mice (*Peromyscus maniculatus*) and kangaroo rats (*Dipodomys ordii* and *D. microps*) can cause disease if parasite numbers become very high (Ernst et al. 1968; Chobotar et al. 1970). Similarly, *T. gondii* infection was rare in woodrats; only three had low antibody titers and a single seronegative woodrat had viable *T. gondii* isolated from tissues (data not shown) (Dubey et al. 2011). This woodrat *T. gondii*-isolate was genetically characterized as a type 12 lineage (Dubey et al. in press). The only previous study on woodrats failed to find any antibodies to *T. gondii* in *N. fuscipes* in California (Dabritz et al. 2008).

Conclusions

Our results provide evidence that southern plains woodrats in this locale have a high diversity of parasites and at least one of the parasites detected, *T. gondii* is zoonotic. A concurrent study also detected a high prevalence of *Trypanosoma cruzi*, another important zoonosis (Charles, unpublished data). Of interest is the absence of gross lesions consistent with *Leishmania mexicana* on any of our woodrats (data not shown) as this parasite has been reported in woodrats, including *N. micropus*, in Texas (McHugh et al., 1990; Grogl et al., 1991; McHugh et al., 2003). Additionally, several of the parasites detected (i.e., *B. neotomofelis*, *T. gondii*, and *Cuterebra*) can cause disease in woodrats. Additionally, the detection of a novel *Babesia*, which has unknown medical or veterinary importance,

highlights the need for further work on parasites or vector-borne pathogens of these common, often peridomestic, rodents.

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References

- Allen KE, Yabsley MJ, Johnson EM, Reichard MV, Panciera RJ, Ewing SA, Little SE. Novel *Hepatozoon* in vertebrates from the southern United States. *J Parasitol*. 2011 in press.
- Anderson SH, Williams ES. Plague in a complex of white-tailed prairie dogs and associated small mammals in Wyoming. *J Wildl Dis*. 1997; 33:720–732. [PubMed: 9391955]
- Baird CR. Incidence of infection and host specificity of *Cuterebra tenebrosa* in bushy-tailed wood rats (*Neotoma cinerea*) from central Washington. *J Parasitol*. 1979; 65:639–644. [PubMed: 512757]
- Baird CR. Bionomics of *Cuterebra austeni* (Diptera: Cuterebridae) and its association with *Neotoma albigula* (Rodentia: Cricetidae) in the southwestern United States. *J Med Entomol*. 1997; 34:690–695. [PubMed: 9439124]
- Boren JC, Lochmiller RL, Boggs JF, Leslie DM Jr. Gastrointestinal helminths of eastern woodrat populations in central Oklahoma. *Southwestern Naturalist*. 1993; 38:146–149.
- Braun JK, Mares MA. *Neotoma micropus*. *Mammalian Species*. 1989; 330:1–9.
- Burkholder JE, Allison TC, Kelly VP. *Trypanosoma cruzi* (Chagas') (Protozoa, Kinetoplastida) in invertebrate, reservoir, and human hosts of the Lower Rio-Grande valley of Texas. *J Parasitol*. 1980; 66:305–311. [PubMed: 6771371]
- Bush AO, Lafferty KD, Lotz JM, Shostak AW. Parasitology meets ecology on its own terms: Margolis et al., revisited. *J Parasitol*. 1997; 83:575–583. [PubMed: 9267395]
- Chandler AC. *Trichuris* species from California rodents. *J Parasitol*. 1945; 31:284–286.
- Charles RA, Ellis AE, Dubey JP, Barnes JC, Yabsley MJ. Besnoitiosis in a southern plains woodrat (*Neotoma micropus*) from Uvalde, Texas. *J Parasitol*. 2011; 97:838–841. [PubMed: 21524194]
- Chobotar B, Anderson LC, Ernst JV, Hammond DM. Pathogenicity of *Besnoitia jellisoni* in naturally infected kangaroo rats (*Dipodomys ordii*) in Northwestern Utah. *J Parasitol*. 1970; 56:195–197.
- Dabritz HA, Miller MA, Gardner IA, Packham AE, Atwill ER, Conrad PA. Risk factors for *Toxoplasma gondii* infection in wild rodents from central coastal California and a review of *T. gondii* prevalence in rodents. *J Parasitol*. 2008; 94:675–683. [PubMed: 18605783]
- Dubey JP, Desmonts G. Serological responses of equids fed *Toxoplasma gondii* oocysts. *Equine Vet J*. 1987; 19:337–339. [PubMed: 3622463]
- Dubey JP, Velmurugan G, Rajendran C, Yabsley MJ, Thomas NJ, Beckmen KB, Sinnott D, Ruid D, Hart J, Fair PA, McFee WE, Shearn-Bochsler V, Kwok OCH, Ferreira LR, Choudhary S, Faria EB, Zhou H, Felix TA, Su C. Genetic characterization of *Toxoplasma gondii* in wildlife from North America revealed widespread and high prevalence of the fourth clonal type. *Int J Parasitol*. 2011; 41:1139–1147. [PubMed: 21802422]
- Dubey JP, Yabsley MJ. *Besnoitia neotomofelis* n. sp. (Protozoa: Apicomplexa) from the southern plains woodrat (*Neotoma micropus*). *Parasitology*. 2010; 137:1731–1747. [PubMed: 20561393]
- Eads RB. The fleas of Texas. Austin, Texas: Texas State Health Department; 1950. p. 85
- Eads RB, Hightower BG. The ticks of Texas, with notes on their medical significance. *Tex J Sci*. 1956; 8:7–24.
- Eads RB, Menzies GC, Miles VI. Acarina taken during west Texas plague studies. *Proc Entomol Soc Wash*. 1952; 54:250–253.

- Ernst JV, Chobotar G, Oaks EC, Hammond DM. *Besnoitia jellisoni* (Sporozoa: Toxoplasmea) in rodents from Utah and California. *J Parasitol.* 1968; 54:545–549. [PubMed: 4990449]
- Fichet-Calvet E, Giraudoux P, Quere JP, Ashford RW, Delattre P. Is the prevalence of *Taenia taeniaeformis* in *Microtus arvalis* dependent on population density? *J Parasitol.* 2003; 89:1147–1152. [PubMed: 14740902]
- Galaviz-Silva L, Mercado-Hernandez R, Ramirez-Bon E, Arredondo-Cantu JM, Lazcano-Villareal D. *Sarcocystis neotomafelis* sp. n (Protozoa; Apicomplexa) from the woodrat *Neotoma micropus* in Mexico. *Rev Latinoam Microbiol.* 1991; 33:313–322. [PubMed: 1670266]
- Grogl M, Kreutzer RD, McHugh CP, Martin RK. Characterization of a *Leishmania* isolate from the rodent host *Neotoma micropus* collected in Texas and comparison with human isolates. *Am J Trop Med Hyg.* 1991; 45:714–722. [PubMed: 1763798]
- Grundmann AW. Cestodes of mammals from the Great Salt Lake desert region of Utah. *J Parasitol.* 1958; 44:425–429. [PubMed: 13564358]
- Gutierrez-Pena EJ. Scanning electron microscopic study of adults and microfilariae of *Dunnifilaria meningica* (Filarioidea: Onchocercidae). *Parasitol Res.* 1989; 75:470–475. [PubMed: 2666981]
- Haas GE, Wilson N, McAllister CT. Fleas (Siphonaptera: Ceratophyllidae, Ctenophthalmidae) from rodents in five southwestern states. *Western North American Naturalist.* 2004; 64:514–517.
- Hall MC. Nematode parasites of mammals of the orders Rodentia, Lagomorpha, and Hyracoidea. *Proc US Nat Mus.* 1916; 50:1–258.
- Hoberg EP, Rickard LG, Zimmerman GL. Synopse of *Nematodirus neotoma* (Trichostrongyloidea). *J Parasitol.* 1988; 74:686–691. [PubMed: 3397830]
- Irizarry-Rovira AR, Wolf A, Bolek M. *Taenia taeniaeformis*-induced metastatic hepatic sarcoma in a pet rat (*Rattus norvegicus*). *J Ex Pet Med.* 2007; 16:45–48.
- Johnson CW. Parasites of *Neotoma micropus* in Zavala County, Texas. *Bull Texas Mem Mus.* 1966; 11:50–55.
- Johnson EM, Allen KE, Panciera RJ, Ewing SA, Little SE, Reichard MV. Field survey of rodents for *Hepatozoon* infections in an endemic focus of American canine hepatozoonosis. *Vet Parasitol.* 2007; 150:27–32. [PubMed: 17942230]
- Keirans JE, Litwak TR. Pictorial key to the adults of hard ticks, family Ixodidae (Ixodida: Ixodoidea), east of the Mississippi River. *J Med Entomol.* 1989; 26:435–448. [PubMed: 2795615]
- Kjemtrup AM, Robinson T, Conrad PA. Description and epidemiology of *Theileria youngi* n. sp from a northern Californian dusky-footed woodrat (*Neotoma fuscipes*) population. *J Parasitol.* 2001; 87:373–378. [PubMed: 11318567]
- Kjemtrup AM, Wainwright K, Miller M, Penzhorn BL, Carreno RA. *Babesia conradae* sp. nov., a small canine *Babesia* identified in California. *Vet Parasitol.* 2006; 138:103–111. [PubMed: 16524663]
- Labruna MB, Whitworth T, Bouyer DH, McBride J, Camargo LM, Camargo EP, Popov V, Walker DH. *Rickettsia bellii* and *Rickettsia amblyommii* in *Amblyomma* ticks from the State of Rondonia, Western Amazon, Brazil. *J Med Entomol.* 2004; 41:1073–1081. [PubMed: 15605647]
- Lewis RE. A taxonomic review of the North American genus *Orchopeas* Jordan, 1933 (Siphonaptera: Ceratophyllidae: Ceratophyllinae). *J Vector Ecol.* 2000; 25:164–189. [PubMed: 11217216]
- Lin YC, Rikihisa Y, Kono H, Gu Y. Effects of larval tapeworm (*Taenia taeniaeformis*) infection on reproductive functions in male and female host rats. *Exp Parasitol.* 1990; 70:344–352. [PubMed: 2311716]
- Linsdale, JM.; Tevis, LP, Jr. The dusky-footed woodrat. Berkeley and Los Angeles, California: University of California Press; 1951. p. 664
- McHugh CP, Grogl M, Kerr SF. Isolation of *Leishmania mexicana* from *Neotoma micropus* collected in Texas. *J Parasitol.* 1990; 76:741–742. [PubMed: 2213421]
- McHugh CP, Thies ML, Melby PC, Yantis LD Jr, Raymond RW, Villegas MD, Kerr SF. Short report: A disseminated infection of *Leishmania mexicana* in an eastern woodrat, *Neotoma floridana*, collected in Texas. *Am J Trop Med Hyg.* 2003; 69:470–472. [PubMed: 14695082]
- McIntosh A. A new dicrocoeliid trematode, *Eurytrema komarek* n. sp. from a white-footed mouse. *Proc Helm Soc Wash.* 1939; 6:18–19.

- Merkelz R, Kerr SF. Demographics, den use, movements, and absence of *Leishmania mexicana* in southern plains of woodrats (*Neotoma micropus*). Southwestern Naturalist. 2002; 47:70–77.
- Miller GE, Schmidt GD. Helminths of bushy-tailed wood rats, *Neotoma cinerea* subsp. from Colorado, Idaho, and Wyoming. Proc Helm Soc Wash. 1982; 49:109–117.
- Noel S, Tessier N, Angers B, Wood DM, Lapointe FJ. Molecular identification of two species of myiasis-causing *Cuterebra* by multiplex PCR and RFLP. Med Vet Entomol. 2004; 18:161–166. [PubMed: 15189241]
- Packchianian A. Reservoir hosts of Chagas' disease in the state of Texas. Natural Infection of nine-banded armadillo (*Dasypus novemcinctus texanus*), house mice (*Mus musculus*), opossum (*Didelphis virginiana*), and wood rats (*Neotoma micropus micropus*), with *Trypanosoma cruzi* in the State of Texas. Am J Trop Med Hyg. 1942; 22:623–631.
- Pinto CM, Baxter BD, Hanson JD, Mendez-Harclerode FM, Suchecki JR, Grijalva MJ, Fulhorst CF, Bradley RD. Using Museum Collections to Detect Pathogens. Emerg Infect Dis. 2010; 16:356–357. [PubMed: 20113586]
- Raun GG. A population of woodrats (*Neotoma micropus*) in southern Texas. Bull Texas Mem Mus. 1966; 11:1–62.
- Sabrosky, CW. North American species of *Cuterebra*, the rabbit and rodent bot flies (Diptera: Cuterebridae). College Park, Maryland: Entomological Society of America; 1986. p. 240
- Santos A III, Tuff DW, Baccus JT. Metazoan parasites of *Peromyscus pectoralis* in central Texas. Tex J Sci. 2010; 62:1–11.
- Schell, SC. Handbook of Trematodes of North America North of Mexico. Moscow, Idaho: University Press of Idaho; 1985. p. 263
- Stark, HE. The Siphonaptera of Utah. Atlanta, Georgia: Their Distribution, Host Relations, and Medical Importance Department of Health, Education, and Welfare, Public Health Service; 1958. p. 239
- Theis JH, Schwab RG. Seasonal prevalence of *Taenia taeniaeformis* - relationship to age, sex, reproduction and abundance of an intermediate host (*Peromyscus maniculatus*). J Wildl Dis. 1992; 28:42–50. [PubMed: 1548801]
- Tucker H. *Nematodirus tortuosus* n. sp. (Neomatoda) from the rat, *Neotoma*. J Parasitol. 1942; 28:159–163.
- Wilson WD, Hnida JA, Duszynski DW. Parasites of mammals on the Sevilleta National Wildlife Refuge, Socorro, New Mexico: *Cuterebra austeni* and *C. neomexicana* (Diptera: Oestridae) from *Neotoma* and *Peromyscus* (Rodentia: Muridae), 1991–1994. J Med Entomol. 1997; 34:359–367. [PubMed: 9151503]
- Yabsley MJ, Davidson WR, Stallknecht DE, Varela AS, Swift PK, Devos JC Jr, Dubay SA. Evidence of tick-borne organisms in mule deer (*Odocoileus hemionus*) from the western United States. Vector Borne Zoonotic Dis. 2005; 5:351–362. [PubMed: 16417431]

Table 1

Oligonucleotide primers used in polymerase chain reaction assays.

Target organism [*]	Gene target	Primer	Primer sequence (5'–3')	Reference
<i>Babesia/Hepatozoon</i> (1°)	18S rRNA	3.1	CTCCTTCCTTTAAGTGATAAG	Yabsley et al. (2005)
<i>Babesia/Hepatozoon</i> (1°)	18S rRNA	5.1	CCTGGTTGATCCTGCCAGTAGT	Yabsley et al. (2005)
<i>Babesia/Hepatozoon</i> (2°)	18S rRNA	RLBH-F	GAGGTAGTGACAAGAAATAACAATA	Yabsley et al. (2005)
<i>Babesia/Hepatozoon</i> (2°)	18S rRNA	RLBH-R	TCTTCGATCCCCTAACTTTC	Yabsley et al. (2005)
<i>Rickettsia</i> (1°)	17kDa antigen	17k-3	TGTCTATCAATTCACAACCTTGCC	Labruna et al. (2004)
<i>Rickettsia</i> (1°)	17kDa antigen	17k-5	GCTTTACAAAATTCTAAAAACCATATA	Labruna et al. (2004)
<i>Rickettsia</i> (2°)	17kDa antigen	17Kd1	GCTCTTGCAACTTCTATGTT	Labruna et al. (2004)
<i>Rickettsia</i> (2°)	17kDa antigen	17kD2	CATTGTTTCGTCAGGTTGGCG	Labruna et al. (2004)
<i>Cuterebra</i>	Cytochrome oxidase I (COI)	C1-J-2183	CAACATTTATTTTGATTTTTTGG	Noël (2004)
		C1-N-2659	GCTAATCCAGTGAATAATGG	
		C2-J-3138	AGAGCTTCACCCTTAATAGAGCAA	
		C2-N-3661	CCACAAATTTCTGAACATTGACCA	

* 1°, primers used in the primary amplification; 2°, primers used in secondary amplification of a nested PCR protocol.

Table 2Helminth parasites of 97 southern plains woodrats (*Neotoma micropus*) from Uvalde County, Texas

Parasite	No. positive (%)	No. collected	Mean intensity	Range
Nematoda				
<i>Trichuris neotomae</i>	78 (80.4)	*448A, 55I	6.4	1–53
<i>Ascarops</i> sp.	42 (43)	100A, 82I	4.3	1–17
<i>Nematodirus neotoma</i>	31 (32)	637	20.5	1–134
Filarial nematodes	7 (7)	n.a.	n.a.	n.a.
<i>Dunnifilaria meningica</i>	4 (4)	n.a.	n.a.	n.a.
Unidentified female spuriid	1 (1)	1	1.0	1
Cestoda				
<i>Raillietina</i> sp.	9 (9)	24	2.7	1–13
<i>T. taeniformis</i> cysts	8(8)	19	24	1–8
Trematoda				
<i>Scaphiostomum</i> sp.	1 (1)	1	1	0–1
<i>Zonorchis</i> sp.	1 (1)	3	3	0–3

*
A, adults; I, immatures

Table 3

Ectoparasite infestation of 104 southern plains woodrats, *Neotoma micropus*, from Uvalde County, Texas.

Ectoparasites	No. collected*	No. infested (%)	Mean intensity	Range
Fleas				
<i>Orchopeas sexdentatus</i>	33♂, 73♀	40 (39)	2.7	1–13
<i>O. neotomae</i>	7♂	6 (10)	1.2	1–2
Ticks				
<i>Ixodes woodi</i>	3A♀, 5N	5 (5)	1.6	1–2
<i>Ornithodoros turicata</i>	1A	1 (1)	1.0	1
Mites				
<i>Trombicula</i> sp.	3L	2 (2)	1.5	1–2
<i>Ornithonyssus</i> (= <i>Bdellonyssus</i>) <i>bacoti</i>	36A	6 (6)	6.0	1–29
Bot-flies				
<i>Cuterebra</i> sp.	15L	7 (12)	2.1	1–4

* L, larva; N, nymphs; A, adults.

Table 4

Bacterial and protozoan parasites of 104 southern plains woodrats (*Neotoma micropus*) from Uvalde County, Texas.

Organism	Diagnostic method	No. infected (%)
<i>Rickettsia</i> spp.	Polymerase Chain Reaction	0 (0)
<i>Babesia</i> sp.	Polymerase Chain Reaction	1 (1)
<i>Hepatozoon</i> sp.	Polymerase Chain Reaction	17 (16)
	Histology	21 (20)
<i>Besnoitia neotomofelis</i>	Gross examination and histology	3 (3)
<i>Sarcocystis neotomafelis</i>	Histology	26 (25)
Coccidian oocysts	Histology	7 (7)
<i>Toxoplasma gondii</i>	Serology	3 (5)