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Full Length Research Paper

Two new flea records from Guatemala: *Pulex simulans* and *Echidnophaga gallinacea* (Siphonaptera: Pulicidae), and their host-parasite relationship

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During bacterial zoonoses studies in 2009 to 2010, two common fleas (*Pulex simulans* and *Echidnophaga gallinacea*) were collected and are reported herein for Guatemala for the first time. Their prevalence, mean intensity and abundance are also presented.

Key words: Guatemala, Siphonaptera, *Pulex simulans, Echidnophaga gallinacea,* opossums, domestic carnivores.

INTRODUCTION

The order Siphonaptera is divided into 16 families and includes 242 genera, and 2005 species (Krasnov, 2008). Although fleas serve as vectors of many zoonotic diseases, together with other hematophagous parasites they also are considered integral parts of the ecosystem, playing a key role on their host species fitness (Durden and Keirans, 1996; Durden and Traub, 2002; Eckerlin, 2006; Krasnov, 2008). There is accumulated evidence that when parasite species diversity increases, ecosystem functioning improves (Hudson et al., 2006). Altizer et al. (2007) demonstrated the negative effects of threatened species on the diversity of their respective parasites (helmints, protozoans, and viruses).

The geographical location between septentrional and meridional hemispheres provides ecological conditions that make Guatemala a mega-diverse country. Mexico and Panama, have reported 138 flea species and 37 species respectively, which could indicate that Guatemala has still many species to report in addition to the 24 species reported so far, since not enough sampling effort has been done (Tipton and Méndez, 1966; Acosta and Morrone, 2003; Eckerlin, 2006). In addition, ecological studies of parasites are powerful tools for understanding the potential spread of dangerous zoonotic diseases, such studies provide a theoretical basis for their control and prevention (Real, 1996; Krasnov, 2008). Species identification and baseline faunistic studies are crucial for control of zoonotic diseases.

We therefore report two common fleas belonging to the family Pulicidae as additions to Guatemala's fauna and the implications and relevance that these unreported fleas might have for public health.

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METHODS

Between March 2009 and October 2010, wild (n=224) and domestic (n=277) mammals were sampled in agricultural landscapes of rural lowlands of Guatemala, Departments of Izabal (northeast), Santa Rosa and Jutiapa (south) (1545.690'N, -88º32.114'W; 14º25.440´N, -90º03.110´W; 14º16.903'N, 89º54.452'W; respectively). These landscapes are dominated by cattle farms, corn, sorghum and tobacco plantations with dispersed young secondary forest remnants. Wild mammals were captured with Sherman and Tomahawk traps (9´×3.5´×3´ H.B. Sherman Traps USA, 32´×10´×12´ Tomahawk Live Trap Company, USA) located in forest edge between 1600 and 1900 h and checked next day at 0800 h. Corn dough, canned tuna and bananas were used as bait. Capture effort was 1962 and 1088 trap/nights, for Sherman and Tomahawk traps, respectively.

Mammals were anesthetized with a combination of xilazine and ketamine chlorhydrate (2 mg/kg, IM) for medium sized mammals, or ketamine chlorhydrate (50 mg/kg, IP), for rodents. Animals were anesthetized and handled by a veterinarian and all capture procedures were according to the Ethics Committee of Universidad del Valle de Guatemala (UVG) and with authorization of the National Protected Areas Council of Guatemala (CONAP). Dogs and cats were sampled at their owner's home after pet owner's consent.

Each animal's entire body was searched for fleas for a minimum of 10 min. Fleas were removed from the body with forceps and then placed in 70% ethanol tagged vials. Samples were transported to the Center of Health Studies of UVG and some were placed in a lactophenol and PVA medium for identification purposes. All fleas were identified according to (Tipton and Méndez, 1966; Acosta and Morrone, 2003). A few samples were fixed with Canada balsam for long term preservation, and are available in the (CES) of UVG at the Bacterial Zoonosis Laboratory.

Flea parameters are presented according to Rózsa et al. (2000) and Reiczigel (2003), namely: mean intensity (MI), mean abundance (MA), and prevalence. Intensity is the number of fleas living on an infected host, and abundance is the number of fleas living on any host (intensity > 0, abundance ≥ 0). Fisher's exact test and Bootstrap-t test are used according to Rózsa et al. (2000), for prevalence and intensity comparisons, respectively.

RESULTS AND DISCUSSION

A total of 2500 fleas were collected, 91 from wild mammals (Didelphidae and Muridae) and 2409 from domestic dogs and cats (Table 1). All fleas belonged to the family Pulicidae, and included: *Ctenocephalides felis* Bouché, 1835 (n=2091); *Echidnophaga gallinacea* Westwood, 1875 (n=318); *Pulex simulans* Baker, 1895 (*n*=61) *Pulex* sp*.* (n=20) and *Ctenocephalides canis* Curtis, 1826 (*n*=10).

All flea species recorded in this study had female-biased sex ratios; such finding is concordant with previous studies about ectoparasites in general (Marshall, 1981) and specifically with studies on fleas (Krasnov, 2008). The reason for such female-bias sex ratio is unclear but may be because females are longer lived than males, because males are more active than females and spend more time off the host, because males are often more prone to predation or grooming by the host, or to male starvation (Marshall, 1981).

Based on examination of the aedeagus at high-magnification (400×) and according to Smit (1958), all *Pulex* sp. male fleas in this study were determined to be *P. simulans*. Therefore, although females of *P. simulans* and *Pulex irritans* cannot be distinguished, we assumed that all *Pulex* sp. females collected were also *P. simulans*, except on those hosts where no male fleas were found. All *Pulex* sp. female fleas (n=20) of hosts where no male fleas where found are reported here as *Pulex* sp. (Table 1).

Pulex simulans is considered a native species to the American continent (New World), and we hypothesize that many alleged reports for Guatemala of *P. irritans* could in fact be specimens of *P. simulans*, considering their strong morphological similitude (Smit, 1958; Layne, 1971; Durden and Traub, 2002; Eckerlin, 2006). In México, *P. simulans* is widely distributed, while *P. irritans* is a rarely reported species; therefore, it is possible that in Guatemala, *P. simulans* has been confused with *P. irritans* and that is the reason is first reported until now (Eckerlin, 2006).

The flea *P. simulans* is capable of transmitting important pathogens and it has shown high infestation rates in homes causing dermatitis in humans (Durden and Traub, 2002). Smit (1958) and Wilson and Bishop (1966) suggested that *P. simulans* was mainly a flea of rodents while *P. irritans* was more associated with big carnivores and humans. Recent studies suggest that *P. simulans* is mainly a flea of carnivores and it was found to be the third more abundant flea on domestic dogs (Durden et al., 2005). However, it is interesting that in Panamá, Central America, *P. simulans* was a rare flea found on domestic dogs (Bermudez and Miranda, 2011).

In this study, *P. simulans* infested rodents, opossums, and domestic dogs, with prevalences and intensities that did not show statistical differences among those three groups (P= 0.482, P= 0.434, respectively). Only one cosmopolitan domestic rodent species was sampled (*Mus musculus*, *n* = 10) and only one *P. simulans* male flea was found on this species. This shows the need to conduct specific sampling of cosmopolitan domestic rodents in rural areas of Guatemala, in order to study their ectoparasites, their ecology and their implications for animal and human health. Three rodent species were parasitized by *Pulex* sp. fleas and only one of those species could be considered to be infested by *P. simulans* (*Sigmodon hispidus*, Table 1). Therefore, we cannot say much about the infestation by *Pulex* spp. (either *P. irritans* or *P. simulans*) on rodents, except that it was the only genus of flea infesting this group of mammals during this study.

Equidnophaga gallinacea was collected on domestic dogs and cats. It showed the highest MI registered for any flea found in this study (MI=15.05) (Table 2), on domestic dogs. This flea is a cosmopolitan medically important pest commonly found on rats, dogs, cats and occasionally on humans (Durden and Traub, 2002; Eckerlin 2006). It had not been reported in Guatemala until now in spite of its conspicuous characteristic angular shaped head, and lack of genal and pronotal ctenidia (Durden and Traub, 2002). The most logical explanation for this could be the scarce publication about fleas from Guatemala that is available in peer reviewed journals, probably due to a low interest of Guatemalan veterinarians in publishing their findings.

The dog flea (*C. canis*) is reported for the third time in Guatemala (Eckerlin, 2006), and its prevalence is very low for this region compared with countries in Asia, where this flea is usually the most reported species (Nithikathkul et al., 2005; Eckerlin, 2006). It was the most rare (MA = 0.02, Prev = 0.08) collected flea during this study.

Table 1. Flea intensity (female/males) per host species of mammals of rural areas of Guatemala, 2009 to 2010.

Order	Family	Mammal species*(n)**	*Ctenocephalides felis*	*Ctenocephalides canis*	*Echidnophaga gallinácea*[&]	*Pulex simulans* [&]	*Pulex* sp. [#]
Carnivora	Canidae	*Canis lupus familiaris* (234)	1259/607	6/4	291/25	16/28	14/0
	Felidae	*Felis catus* (43)	104/53		2/0		
Cingulata	Dasypodidae	*Dasypus novemcinctus* (1)					
Rodentia	Muridae	*Heteromys* sp. (13)				2/2	
		Mus musculus (10)				0/1	
		Sigmodon hispidus (11)				4/2	1/0
		Zygodontomy s sp. (2)				0/1	1/0
Didelphimorphia	Didelphidae	*Didelphis marsupialis* (45)	24/7			0/2	3/0
		Didelphis virginiana (34)	16/13			2/1	1/0
		Philander opossum (35)	3/5				

* Amount of mammal captured. [&] First reports for Guatemala. # *Pulex* sp. female fleas cannot be identified to species (Smit, 1958). Therefore, if no male fleas were found on an infested host, *Pulex* sp. female fleas could be either *P. simulans* or *P. irritans*.

Table 2. Infestation parameters of fleas collected from domestic and wild mammals in rural areas of Guatemala, 2009 to 2010.

Flea and host	% Prevalence (infested)	95%[&] Confidence interval	Mean intensity	95%[#] Confidence interval	Mean abundance	95%[#] Confidence interval
Ctenocephalides felis	45.7 (229)	41.32 - 50.00	9.13	7.68 - 10.97	4.17	3.44 - 5.19
Cats	79.1 (34)	64.10 - 88.90	4.62	3.47 - 5.88	3.65	2.60 - 4.88
Dogs	70.1(164)	63.91 - 75.67	11.38	9.49 - 13.93	7.97	6.62 - 9.94
Opossums (*Philander oposum, Didelphis marsupialis and D. virginiana*)	27.2 (31)	19.65 - 36.35	2.19	1.68 - 2.87	0.60	0.39 - 0.89
***Pulex simulans* ***	4.8 (24)	3.16 - 7.05	2.67	1.96 - 4.17	0.13	0.07 - 0.22
Rodents	11.1 (4)	3.89 - 26.08	2.75	1.00 - 3.50	0.31	0.08 - 0.69
Dogs	6.8 (16)	4.20 - 10.84	2.94	1.94 - 5.38	0.21	0.10 - 0.41
Opossums	5.1 (4)	1.75 - 12.46	1.50	1.00 - 2.00	0.08	0.01 - 0.19
***Echidnophaga gallinacea* ***	4.6 (23)	3.05 - 6.85	13.83	5.52 - 36.13	0.63	0.22 - 1.86
Dogs	9.0 (21)	5.86 - 13.40	15.05	6.19 - 39.67	1.35	--
Cats	4.7 (2)	--	1.00	--	1.00	--
Ctenocephalides canis	0.8 (4)	0.28 - 2.05	2.50	1.00 - 5.00	0.02	0.00 - 0.06
Dogs	1.7 (4)	0.59 - 4.39	2.5	1.00 - 5.00	0.04	0.01 -0.14

* First reports for Guatemala; [&] confidence limits using the Sterne's exact method, according to Reiczigel (2003); [#] bootstrap (*BCa*) confidence limits, according to Rózsa et al. (2000).

The cat flea (*C. felis*) is a well known reservoir of pathogens (example, *Rickettsia felis)* that could affect both humans and animals in Central America (Bermúdez et al., 2011; Hun et al., 2011). This flea was the most abundant flea during this study (MA = 4.17) and it was more abundant on dogs (MA = 7.97) than on all other infested mammal species (opossums and domestic cats, MA ≤ 3.65). The highest MI and prevalence of *C. felis* were registered on domestic dogs (MI = 11.38) and cats (Prevalence= 0.701), respectively (Table 2).

Domestic carnivores, and opossums, are hosts for the most abundant and opportunistic fleas reported for this study (*E. gallinacea*, *C. felis* and *P. simulans*). We therefore recommend more research related to the ectoparasites of wild and domestic mammals in rural areas of Central America. Additionally, the community structure and ecology of rodents, opossums and domestic carnivores (specifically dogs and cats), and their host-parasite dynamics require further study to understand their real implications in human and animal health.

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