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LOUSE-BORNE BACTERIAL PATHOGENS IN LICE (PHTHIRAPTERA) OF RODENTS AND CATTLE FROM EGYPT

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ABSTRACT: We collected 1,023 lice, representing 5 species, from rats and domestic cattle throughout 13 governorates in Egypt and tested these lice for *Anaplasma marginale*, *Bartonella* spp., *Brucella* spp., *Borrelia recurrentis*, *Coxiella burnetii*, *Francisella tularensis*, and *Rickettsia* spp. by PCR amplification and sequencing. Five different louse-borne bacterial agents were detected in lice from rodents or cattle, including "*Bartonella rattimassiliensis*", "*B. phoceensis*", and *Bartonella* sp. near *Bartonella tribocorum*, *Coxiella burnetii*, and *Rickettsia typhi*. More lice from governorates bordering the Mediterranean and Red Seas contained pathogens. Our data indicate that lice of urban and domestic animals harbor pathogenic or potentially pathogenic bacterial agents throughout Egypt.

Body lice and their associated louse-borne pathogens have been a scourge to humans throughout history. Louse control, following the invention of synthetic pesticides, modern laundering technology, and antibiotics greatly reduced the prevalence of these parasites and the diseases they transmit. Wild and domestic mammals continue to harbor lice, which can serve as reservoirs or vectors of pathogens to humans. Lice are the vectors or intermediate hosts of *Anaplasma* spp., *Bartonella quintana*, *Borrelia recurrentis*, *Brucella* spp., *Francisella tularensis*, *Rickettsia prowazekii*, *R. typhi*, swinepox virus, dog tapeworm, and filarial nematodes (Reiss-Gutfreund, 1966; Morsy, Fayad et al., 1986; Durden, 2002). Lice have plagued humans for centuries, and louse-borne diseases decimated troops in every major European war or emerged to slaughter survivors of natural and man-made disasters (Szybalski, 1999; Raoult, Woodward et al. 2004). For example, epidemic typhus killed more people throughout history than the sum of all those who died in combat since the Peloponnesian wars (Raoult, Woodward et al. 2004). An epidemic of louse-borne relapsing fever killed more than 50,000 people in Africa and Europe during World War II (Borgnolo et al. 1993).

Lice and louse-borne pathogens have been problems in Egypt since the time of the pharaohs (Exodus 8:16–17). Epidemic typhus was widespread in Egypt following World Wars I and II, but prevalence of the disease dramatically decreased after the introduction of DDT for louse control (Taylor, 1957). Body lice, *Pediculus humanus* Linnaeus, remain a public health concern in Egypt (Morsy, El-Ela et al. 2001), but epidemic typhus has not been a significant public health problem in recent years. The Egyptian population is at risk for epidemics of trench fever, louse-borne relapsing fever, or epidemic typhus, because neighboring African countries with refugee populations have ongoing epidemics of these diseases (e.g., Raoult, Ndihokubwayo et al. 1998; Raoult and Roux, 1999; Ramos et al. 2004; Mokrani et al. 2004), and human populations in cities such as Cairo often live in close proximity to rodents and feral animals (Daniel et al. 1989). Several other pathogens transmitted by lice, but generally considered to be transmitted by other routes, such as tularemia, murine typhus, and brucellosis, have been reported in Egypt in the past (Zaki, 1965; Trevisanato, 2004).

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Peridomestic rodents, such as *Rattus norvegicus* (Berkenhout) and *R. rattus* (Linnaeus), are reservoirs of arthropod-borne bacterial agents that are pathogenic to humans (Ellis et al., 1999; Comer, Diaz et al. 2001). Rodents have been incriminated as reservoirs of bacterial pathogens in Egypt (Imam and Salah, 1966). However, no detailed survey of the bacterial agents in rodent lice from Egypt has been conducted. Surveys of the louse fauna of Egypt revealed that *Polyplax spinulosa* (Burmeister) is widely distributed on *Rattus* spp. throughout the country (Johnson 1960a; Gaaboub et al. 1982; Morsy, Fayad et al. 1986; Shoukry, Morsy et al. 1986; Shoukry and Farahat 1987; Soliman et al. 2001). Additional species of lice, such as *Hoplopleura pacifica* Ewing, infest *Rattus* spp. throughout the world (Durden and Musser, 1994) and should be present in Egypt. Johnson (1960a) did not report *H. pacifica* from *Rattus* spp. in her key to the lice of Egyptian rodents, and subsequent identifications of a louse of mice, *Hoplopleura capitosa* Johnson, from *Rattus* spp. in Egypt, are undoubtedly erroneous (e.g., Gaaboub et al. 1982). Lice of other domestic African animals, including *Haematopinus* spp. and *Linognathus* spp. (e.g., Gabaj et al. 1993), are present in Egypt. We initiated a study to determine if lice of peridomestic rodents and domestic animals in Egypt harbored pathogenic bacterial agents.

MATERIALS AND METHODS

Rodents, such as *R. rattus* and *R. norvegicus*, were collected using live traps set for 2 successive nights at each site. A range of 30–50 wire traps of the spring-door type were used, and fresh fruit, vegetables, and peanut butter wrapped in gauze were used as bait. Traps were set in the evening, inside and outside of houses and animal shelters, and were checked the following morning. Trapped rodents were anesthetized with the use of an ether-charged chamber and identified with the use of keys by Morsy, Michael et al. (1982) and Osborn and Helmy (1980). Species of *Rattus* were killed. All other animals were released.

Ectoparasites were brushed off each animal and fixed in 70% ethanol. Representative lice were cleared in hot 85% lactic acid and slide mounted for microscopic examination. Lice were identified with the use of taxonomic keys by Johnson (1960a, 1960b) and Kim et al. (1986). Voucher specimens of each louse taxon were deposited in the Institute of Arthropodology and Parasitology collection at Georgia Southern University, Statesboro, Georgia. Accession numbers are as follows: *Haematopinus eurysternus* (Nitzsch) (L3296, L3297, L3298), *Haematopinus quadripertusus* Fahrenholz (L3299), *Hoplopleura pacifica* (L3302), *Polyplax spinulosa* (L3300, L3303, L3304, L3305), and *Pediculus humanus* Linnaeus (head of louse only) (L3301).

Individual adults of *Haematopinus* spp. or pools of conspecific lice (5 nymphs of *H. eurysternus* or all polyplacid or hoplopleurid lice from each animal per pool) (Table I) were frozen in liquid nitrogen and crushed with a sterile Teflon pestle. Pulverized lice were incubated with

TABLE I. Collection data and bacterial agents identified from lice collected in Egypt from August 2002 to June 2003.

Species of louse	Collection site	Date	Host	Number of lice/life stage/pools			Bacterial agents detected
Cairo Governate							
Polyplax spinulosa	Garbage Village (Mokattam Village Cairo)	8 August 2002	*Rattus norvegicus*	1	N	1	None
Pediculus humanus	Garbage Village (Mokattam Village Cairo)	8 August 2002	*R. norvegicus*	1	A	1	None
Wadi El Natroun Governate							
P. spinulosa	Hay El Zehor	13 August 2002	*Rattus rattus*	7	B	3	None
Ismailia Governate							
Haematopinus eurysternus	El Warsha	27 August 2002	*Bos taurus*	64	B	27	*Coxiella burnetii* 1/27 pools
Haematopinus eurysternus	Abo Kharwa	27 August 2002	*Bos taurus*	15 5	B	13 1	None
Haematopinus quadripertusus	Abo Kharwa	27 August 2002	*Bos taurus*	22	A	22	None
Suez Governate							
Hoplopleur a pacifica	Arab El Maamal	23 October 2002	*R. rattus*	39	B	8	None
P. spinulosa	Arab El Maamal	23 October 2002	*R. rattus*	25	B	4	None
Sharquiia Governate							
P. spinulosa	Sheeba Zagazig	30 October 2002	*R. rattus*	23	B	4	None
P. spinulosa	Manshiit El Sadat Zagazig	31 October 2002	*R. rattus*	15	B	3	None
P. spinulosa	Qasr Ahmed Salem	31 October 2002	*R. rattus*	5	B	2	None
P. spinulosa	Mit Zaser	31 October 2002	*R. rattus*	1	N	1	None
Alexandria Governate							
P. spinulosa	Fishermen village	12 November 2002	*R. norvegicus*	91	B	13	"*Bartonella rattimassiliensis*" 5/13 pools
H. pacifica	Fishermen village	12 November 2002	*R. norvegicus*	45	B	6	"*B. rattimassiliensis*" 1/6 pool, "*Bartonella phoceensis*" and "*B. rattimassiliensis*" 4/6 pools
Port Said Governate							
P. spinulosa	Exbit Abo Souf	26 November 2002	*R. norvegicus*	18 9	B	8	"*B. rattimassiliensis*" 2/8 pools
Dakahilia Governate							
P. spinulosa	Kolangil Mansoura	2 December 2002	*R. rattus*	4	A	2	None
Matrouh Governate							
P. spinulosa	Tabia	18 December 2002	*R. rattus*	5	N	2	None
P. spinulosa	West village	19 December 2002	*R. rattus*	32	B	3	None
P. spinulosa	Qara Oasis	24 January 2003	*R. rattus*	14	B	6	None

TABLE I. Continued.

Species of louse	Collection site	Date	Host	Number of lice/life stage/pools			Bacterial agents detected
Red Sea Governate							
P. spinulosa	El Qusier	20 March 2003	*R. norvegicus*	30	B	3	None
P. spinulosa	El Malah Hurghada	3 February 2003	*R. rattus*	11	B	5	"*B. rattimassiliensis*" 1/5 pools
P. spinulosa	Souk Safaga	4 February 2003	*R. rattus*	26	B	11	"*B. rattimassiliensis*" 1/11 pools
H. pacifica	Souk Safaga	4 February 2003	*R. rattus*	3	B	2	None
P. spinulosa	Barahima Safaga	5 February 2003	*R. norvegicus*	3	B	2	None
H. pacifica	Barahima Safaga	5 February 2003	*R. norvegicus*	1	A	1	*Rickettsia typhi* 1 louse
P. spinulosa	El Arab Hurghada	2 February 2003	*R. rattus*	2	A	2	None
Aswan Governate							
P. spinulosa	Fish factory	20 April 2003	*R. norvegicus*	4	B	2	None
P. spinulosa	Hagaroub	21 April 2003	*R. norvegicus*	2	N	1	None
Fayoum Governate							
P. spinulosa	Ebshaway	16 June 2003	*R. rattus*	4	A	2	None
P. spinulosa	Esta	16 June 2003	*R. rattus*	1	A	1	*Bartonella* near *tribocorum* 1 louse

A = adult lice, N = nymphal lice, B = both adult and nymphal lice.

220 µl of a lysis buffer containing 1 mg/ml of Proteinase K (VWR, West Chester, Pennsylvania) for 2 hr at 55 C. Lysed samples were transferred to a 96-well DNA binding plate and extractions were completed with the use of a Biomek 2000 Laboratory Automation Workstation (Beckman, Fullerton, California). DNA extraction was performed with the use of a Promega Wizard SV96 Genomic DNA Purification System (Promega, Madison, Wisconsin). DNA was eluted from the membranes into sterile nuclease-free polypropylene 96-well plates with the use of 100 µl of nuclease-free water.

Five hundred twenty-eight lice were screened for DNA from bacterial agents by polymerase chain reaction (PCR) amplification. We used the External forward, External reverse (Shimada et al. 2004); QHEV1, QHEV4 (Houpikan and Raoult, 2001); FlaLL, FlaRL, FlaLS, FlaRS (Barbour et al. 1996); bruc1, bruc5 (Bogdanovich et al. 2004); and FNA8L, FNB2L, FNA7L, FNB2L (Fulop et al. 1996) PCR primers to screen for DNA from *Anaplasma marginale*, *Bartonella* spp., *Borrelia* spp., *Brucella* spp., and *Francisella tularensis*, respectively, with the use of previously published PCR conditions. *Bartonella* spp. were further characterized by PCR amplification of *rib*C, *fts*Z, and *gro*EL genes with the us of the primers described by Johnson et al. (2003), Sanogo et al. (2003), and Marston et al. (1999). Positive and negative controls were used and consisted of genomic DNA extracts of bacterial agents tested for or distilled water. PCR products were separated by 2% agarose gel electrophoresis and visualized under ultraviolet light with ethidium bromide. Products were purified with a QIAquick PCR Purification Kit (Qiagen, Valencia, California). Duplicate sequencing reactions were performed with a BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, California) using PCR primers, and excess dye was removed with a DyeEx 2.0 column (Qiagen). Sequences were determined using an ABI 3100 capillary sequencer (Applied Biosystems). Primer sequences were removed and sequences assembled with Seqmerge (Accelrys, San Diego, California). Assembled sequences were compared to those in GenBank with the use of the BLAST 2.0 program (NCBI, Bethesda, Maryland). Identification of bacterial species was based on sequence similarity to known species.

DNA extracts were screened for *Coxiella burnetii* and *Rickettsia* spp. With the use of real-time PCR. A Biomek 2000 Laboratory Automation Workstation (Beckman, Fullerton, California) prepared reactions in 384-well plates, with 2.0 µl of template DNA in a 20 µl final reaction volume. PCR amplification and data analysis were performed with the use of a 7900HT thermocycler and associated software (Applied Biosystems). A Brilliant qPCR Core Reagent Kit (Stratagene, La Jolla, California) was used for TaqMan assays, which use a fluorescent oligonucleotide probe. The IS1111 transposable element of *Coxiella burnetii* was detected with the use of a TaqMan assay (IS1111F 5′-CCGATCATTTGGGCGCT-3′ 1600 n*M*, IS1111R 5′-CGGCGGTGTTTAGGC-3′ 800 n*M*) with 200 n*M* of a FAM-labeled probe (5′-FAM-TTAACACGCCAAGAAACGTATCGCTGTG-3′). The 17-kD antigenic gene of *Rickettsia* spp. was detected with the use of primers described by Jianget al. (2004), with 100 n*M* of a newly designed probe (5′-FAM-TTGGTTCTCAATTCGGTAAGGGTAAAGG-3′). Both assays use the same thermocycler conditions: 95 C for 10 min, followed by 40 cycles of 95 C for 15 sec and 60 C for 60 sec. Samples positive for *Rickettsia* were further analyzed with the use of conventional PCR to amplify the 17-kD antigenic gene of *Rickettsia* spp. (Carl et al. 1990) and sequenced as previously described.

RESULTS

Five species of lice were collected from rodents and cattle throughout Egypt, and DNA from *Bartonella* spp., *R. typhi*, and *C. burnetii* were detected by real-time or conventional PCR and sequencing (Table I). Three species of *Bartonella* were detected in lice of rodents. We detected "*B. rattimassiliensis*" and "*B. phoceensis*" in pools of lice from 3 governorates adjacent to the Mediterranean and Red Seas (Table I). The third species of *Bartonella*, with a 99% sequence similarity to the *groEL* gene of *B. tribocorum*, was detected in an adult *P. spinulosa* from *R. rattus* collected on 16 June 2003 in Esta, Fayoum Governorate. DNA from *R. typhi* was detected in an adult *H. pacifica* from a *R. norvegicus* collection on 5 February 2003 in Barahima, Safaga, Red Sea Governorate. *Coxiella burnetii*, the agent of Q fever, was detected in a pool of 5 nymphs of *H. eurysternus* from a cow in El Warsha, Ismailia Governorate.

DISCUSSION

The most frequently collected louse on *Rattus* spp. was *P. spinulosa*, followed by *H. pacifica*. A single specimen of *P. humanus* was recovered from a *R. norvegicus* in the Mokattam Garbage Village, Cairo. This louse is an ectoparasite of humans and undoubtedly represents a contaminant from infested humans present when the trap was set. The collection of 5 *P. spinulosa* from *Meriones libycus* Lichtenstein implies contamination of the trap by lice from previously trapped rats. Two species of lice were collected from cattle. The most frequently collected cattle louse was *H. eurysternus*, but *H. quadripertusus* was collected from a cow in Abo Kharwa, Ismailia Governorate.

Bartonella tribocorum infects *R. norvegicus* and is closely related to *B. elizabethae*, an agent of endocarditis in humans (Heller et al. 1998). Amplification of the *rib*C and *gro*EL genes were successful only from lice harboring ''*B. phoceensis*'', but the *ftsZ* and QHEV1 and QHEV4 primers amplified DNA from all species. With the exception of a single base mismatch for the *B. tribocorum* sequence (GenBank accession number AF304018), all sequences were 100% matches to those in GenBank for the *gro*EL gene of ''*B. phoceensis*'' (e.g., AY515129) and for the *fts*Z gene of ''*B. rattimassiliensis*'' (e.g., AY515133).

Bartonella spp. are Gram-negative bacteria that infect the erythrocytes of vertebrates and are putatively transmitted by blood-feeding arthropods (e.g., Comer, Paddock, and Childs, 2001). At least 9 species of *Bartonella* have been associated with disease in humans (Ciervo and Ciceroni, 2004; Dehio et al. 2004). Of these pathogenic *Bartonella* spp., most appear to be opportunistic zoonotic pathogens with the exceptions of *B. bacilliformis* and *B. quintana*, for which humans are the only reservoirs identified. The *Bartonella* spp. of rodents include several species that are pathogenic to humans (Ellis et al. 1999; Breitschwerdt and Kordick, 2000; Comer, Paddock, and Childs, 2001). The arthropod vectors and transmission cycles for most *Bartonella* spp. are unknown, and the vectors of *Bartonella* spp. among peridomestic rodents might include lice. *Pediculus humanus*, the human body louse, is the primary vector of *B. quintana*. The vectors for *Bartonella* spp. in most wild rodents are not known.

Gundi et al. (2004) failed to incriminate fleas as vectors of ''*B. rattimassiliensis*'' or ''*B. phoceensis*'' among *Rattus* spp. in France. They tested fleas from infected rats for both *Bartonella* spp., but were unable to detect these agents in fleas. Lice were not examined in regards to the transmission of these agents. They claimed that their rats were free of all other ectoparasites, but did not describe collection techniques that would collect lice or mites. Our data suggest that both ''*Bartonella phoceensis*'' and ''*B. rattimassiliensis*'' are associated with lice. ''*Bartonella phoceensis*'' was detected only in *H. pacifica* from rats that were also harboring ''*B. rattimassiliensis*''. However, ''*B. rattimassiliensis*'' was detected from both *P. spinulosa* and *H. pacifica*. Our data could indicate that these *Bartonella* spp. interact with their vectors or rodent hosts and that the presence of one agent is associated with the other. In addition, our data support the hypothesis that ''*B. phoceensis*'' might be associated with or transmitted by *H. pacifica* and not *P. spinulosa*. Both species of lice might be associated with the transmission of ''*B. rattimassiliensis*'', which was the most frequently detected species of *Bartonella*. Experimental transmission studies using *Rattus* spp. and rodent lice are needed to demonstrate louse-borne transmission of these agents. Because *Rattus* spp. and their lice can be maintained in the laboratory, these *Bartonella* species could be model systems for studies of louse-borne pathogens. Further field studies of rat populations harboring these pathogens could focus on randomly sampled rodents and determinations of the minimum field infection rates of these agents.

The public health threats of these *Bartonella* spp. are unknown, but should be assessed. These bacteria are potential pathogens and are associated with urban rodents. Some *Bartonella* spp. of *Rattus* spp. are pathogenic to humans. The louse-associated *Bartonella* spp. of rodents are widespread in Egypt, and we detected DNA in 15 pools of lice from 4 governorates. Further study of undiagnosed or culture-negative bacterial diseases in Egyptians could implicate these agents. Rodents will continue to live in close proximity to humans and, therefore, human exposure to several rodent-associated species of *Bartonella* is likely to continue.

The role of lice in the transmission cycle of *R. typhi* is poorly defined. Both *P. spinulosa* and *H. pacifica* have been implicated in the transmission of *R. typhi*, but Traub et al. (1978) indicated only *H. pacifica* was a vector, and older identifications of *P. spinulosa* were erroneous. The oriental rat flea, *Xenopsylla cheopis* Rothschild, is considered to be the primary vector of *R. typhi* and maintains this pathogen by both vertical and horizontal transmission (Farhang-Azad et al. 1985). Neither *H. pacifica* nor *P. spinulosa* are known to feed on humans; therefore, these lice might be poor bridge vectors of murine typhus to humans. Lice are overlooked as potential enzootic vectors of *R. typhi*, and rodents can harbor more lice than fleas. Transmission of *R. typhi* to vertebrates involves inhalation of infected arthropod feces or scratching infected arthropods or their feces into the skin. Lice might produce infected feces and whether wild rats acquire *R. typhi* from fleas or lice is unknown. Humans living in rat-infested buildings could inhale louse feces and acquire *R. typhi*. A similar mechanism has been proposed to explain the transmission of *R. prowazekii* from the ectoparasites of flying squirrels to humans in the United States (McDade, 1987).

Coxiella burnetii is an obligate intracellular bacterium that infects macrophages, but the agent can be shed in milk and urine (Thiele et al. 1994; McQuiston and Childs, 2002). Most cases of Q fever in humans are acquired from domestic ruminants or their byproducts, but ticks and other arthropods might play roles in transmitting *C. burnetii* (McQuiston and Childs, 2002). Historically, Q fever has been a disease of humans and domestic animals throughout Egypt and northern Africa (e.g., Mooser et al. 1961; McDade et al. 1973; Botros et al. 1995), and detection of DNA from *C. burnetii* in a pool of lice from a cow is not unexpected.

More lice from governorates bordering the Mediterranean and Red Seas contained pathogens. Five different bacterial agents were detected in lice from rodents or cattle sampled throughout Egypt. Neither *C. burnetii* nor *R. typhi* are transmitted primarily by lice, but louse-borne transmission of these agents is possible. The vectors of *Bartonella* spp. of rodents are poorly studied, and lice could serve as the primary vectors of these agents. Lice of rodents do not feed on humans, which makes louse-borne transmission of these agents to humans improbable. If lice are the primary vectors of the *Bartonella* spp. of rodents, and if these *Bartonella* spp. are pathogenic to humans, then the paucity of human cases could be explained by a lack of a suitable bridge vector. Neither *Anaplasma marginale*, *Brucella* spp., *B. recurrentis*, nor *F. tularensis* were detected by PCR in any of the lice. With the exception of *B. recurrentis*, lice are not the primary vectors of these agents. Our failure to detect these pathogens was not surprising, but these agents could still be present in Egypt.

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