

SURVEILLANCE OF EGYPTIAN FLEAS FOR AGENTS OF PUBLIC HEALTH SIGNIFICANCE: *ANAPLASMA*, *BARTONELLA*, *COXIELLA*, *EHRlichia*, *RICKETTSIA*, AND *YERSINIA PESTIS*

AMANDA D. LOFTIS,* WILL K. REEVES, DANIEL E. SZUMLAS, MAGDA M. ABBASSY, IBRAHIM M. HELMY,
JOHN R. MORIARITY, AND GREGORY A. DASCH

Viral and Rickettsial Zoonoses Branch, Centers for Disease Control and Prevention, Atlanta, Georgia; Navy Disease Vector Ecology and Control Center, Jacksonville, Florida; Vector Biology Research Program, NAMRU-3, Cairo, Egypt

Abstract. Serologic surveys in Egypt have documented human and animal exposure to vector-borne bacterial pathogens, but the presence and distribution of these agents in arthropods has not been determined. Between July 2002 and July 2003, fleas were collected from 221 mammals trapped in 17 cities throughout Egypt. A total of 987 fleas were collected, representing four species (*Ctenocephalides felis*, *Echidnophaga gallinacea*, *Leptopsylla segnis*, and *Xenopsylla cheopis*); 899 of these fleas were *X. cheopis* from rats (*Rattus* spp.). Fleas were tested for DNA from *Anaplasma* spp., *Bartonella* spp., *Coxiella burnetii*, *Ehrlichia* spp., *Rickettsia* spp., and *Yersinia pestis*. *Rickettsia typhi*, the agent of murine typhus, was detected in *X. cheopis* and *L. segnis* from rats from nine cities. A spotted-fever group *Rickettsia* sp. similar to “RF2125” was detected in *E. gallinacea*, and two unidentified spotted fever group *Rickettsia* were detected in two *X. cheopis*. Novel *Bartonella* genotypes were detected in *X. cheopis* and *L. segnis* from three cities. *Coxiella burnetii* was detected in two fleas. *Anaplasma*, *Ehrlichia*, and *Y. pestis* were not detected.

INTRODUCTION

Historical epidemics in Egypt include several reports of vector-borne bacterial agents, such as epidemic typhus, murine (endemic) typhus, and bubonic plague. The Justinian plague pandemic of AD 542 originated in Egypt, and epidemics were reported in Egypt until the middle of the 20th century.^{1,2} Outbreaks of typhus occurred in 1915–1920 and 1942–1945; in 1942, > 23,000 human cases of typhus were recorded in Egypt.³ During the 1950s, *Coxiella burnetii*, the agent of Q fever, was isolated from humans, domestic ruminants, and ticks, and at least 20% of the human population was seropositive for this pathogen.⁴ Significant decreases in morbidity from typhus and plague have been attributed to rodent control programs that were initiated in 1942 and to DDT dusting of human populations, which was initiated in 1946.^{2,5} Ten years later, the prevalence of typhus group antibodies in the population was 18%.⁵ Most of the seropositive people had low antibody titers, consistent with previous infection; of the 50 cases with serologic evidence supporting a recent infection, 35 were attributed to epidemic typhus (*Rickettsia prowazekii*) and 15 to murine typhus (*Rickettsia typhi*). In 1963, serologic results suggested that 15% of the undifferentiated febrile illnesses in Egypt were caused by typhus infections.⁶

More recently, serologic surveys of humans, rodents, and domestic animals have documented the continued presence of rickettsial agents in Egypt, including *Bartonella*, *Coxiella*, *Ehrlichia*, and spotted fever and typhus group *Rickettsia*. Serosurveys have revealed that 10–30% of adult blood donors, healthy school children, and febrile illness patients have been exposed to *C. burnetii*, suggesting that the prevalence of antibodies against this agent has changed little since the 1950s.^{7–9} Serologic reactivity against spotted fever group *Rickettsia* (SFGR) has been assessed using *Rickettsia conorii* antigen, and antibodies have been detected in both humans and rodents, including *Acomys* sp., *Gerbillus* sp., and *Rattus*

rattus.^{8,10} Botros and others¹⁰ reported human seroreactivity to typhus group *Rickettsia* (TGR) in 19% of 178 garbage collectors and rodent control workers from five governorates in northern Egypt and in 33% of 109 patients with febrile illness. In a community-based study in the Nile River Delta, TGR seroprevalence approaching 50% in both adults and children was reported.^{8,11} In a larger survey, antibodies reactive with *R. typhi* were detected in 48% of 976 Egyptian patients with acute febrile illness between 1998 and 2000, with the highest seroprevalence rates in the Nile River Delta and Cairo (M.G. Reynolds, personal communication). Rodents are reservoirs of murine typhus, and serologic reactivity of rodents to TGR has been reported throughout Egypt in *Acomys* sp., *Arvicanthis niloticus*, *Gerbillus* sp., *Hemiechinus auritus*, *Meriones* sp., *Mus musculus*, *Rattus norvegicus*, and *R. rattus*.^{12–15} In Egypt, antibodies against TGR have also been detected in buffalo, camels, dogs, donkeys, foxes, goats, and sheep.^{13,15–17} Serosurveys of *Bartonella* and *Ehrlichia* in the human population of Egypt have not been published, but antibodies reactive with *Bartonella henselae* and *B. quintana* were detected in sera from domestic cats,¹⁸ and antibodies reactive with *Ehrlichia canis* were detected in 33% of a study population of domestic dogs.⁷

Although the persistence of vector-borne bacterial agents in Egypt has been documented by human disease reports and by serologic evidence, little data are available on the presence of these agents in the ectoparasitic fleas that might transmit the diseases from animals to humans. Collections of fleas from peridomestic mammals in governorates throughout Egypt have included *Ctenocephalides felis*, *Echidnophaga gallinacea*, *Leptopsylla segnis*, *Nosopsyllus fasciatus*, *Pulex irritans*, *Xenopsylla cheopis*, and *Xenopsylla ramesis*.^{16,19–26} Of these, *X. cheopis*, the primary vector of plague and murine typhus, was the most common flea in all collections. *Leptopsylla segnis*, an experimental vector of murine typhus,²⁷ and *E. gallinacea*, which can be naturally infected with murine typhus,²⁸ were also commonly reported fleas. We report the collection, identification, and polymerase chain reaction (PCR)-based bacterial pathogen testing of fleas from peridomestic, urban mammals from 17 cities in Egypt.

* Address correspondence to Amanda D. Loftis, CDC, 1600 Clifton Road NE, MS G-13, Atlanta, GA 30333. E-mail: aloftis@cdc.gov

MATERIALS AND METHODS

Mammal and flea collection. Animals and fleas were collected from 17 cities in Egypt between July, 2002, and July 2003. All aspects involving animal use were conducted in accordance with the Animal Welfare Act implementing instructions (9 CFR, Subchapter A, Parts 1–3), Department of Defense regulations, and recognized standards relating to the care and use of laboratory animals (NAMRU-3 Animal Protocol Number 02-05). Small and medium-sized mammals were collected using wire, spring-door type live traps baited with fresh fruit, vegetables, and peanut butter wrapped in gauze. Thirty to 50 traps were set up for two or three nights at each site, and traps were placed inside and outside of houses and near animal shelters. Traps were checked each morning, trapped animals were anesthetized with ether, and animals were identified using reference keys by Morsy et al. (1982)²⁹ and Osborn and Helmy (1980).³⁰ Ectoparasites were brushed off each animal and placed into 70% ethanol. Rodents of the genera *Rattus* and *Mus* were humanely euthanized; all other animals were released unharmed. Fleas were identified using keys by Lewis (1967)³¹ and Hoogstraal and Traub (1965).³² Prior to DNA extraction, the gender, species, and host animal for each flea were recorded. Voucher specimens were deposited at the Georgia Museum of Natural Sciences, Entomology Museum, University of Georgia, Athens, GA.

DNA extraction and pooling strategy. Extraction of DNA from individual fleas was performed as described by Moriarity and others,³³ using a Wizard SV96 Genomic DNA Purification System (Promega, Madison, WI), and samples were eluted into sterile, nuclease-free polypropylene 96-well plates. The yield of eluted DNA using this procedure is 50–60 µL/arthropod (average, 55 µL).³³ After extraction, 10 µL aliquots of eluted DNA from each flea were combined, in separate plates, to yield pools representing three fleas each. All DNA samples were stored at 4°C.

Real-time PCR assays. Pooled and individual DNA samples were tested, singly or in duplicate, respectively, using real-time PCR. A Biomek 2000 Laboratory Automation Workstation (Beckman, Fullerton, CA) prepared reactions in 384-well plates, with 1.0 µL of template DNA in a 10-µL final reaction volume, and PCR amplification and data analysis were performed using a 7900HT thermocycler and associated software (Applied Biosystems, Foster City, CA). The Brilliant qPCR Core Reagent Kit (Stratagene, La Jolla, CA) was

used for TaqMan assays, which use a fluorescent oligonucleotide probe. The SYBR Green PCR Core Reagent kit (Applied Biosystems) was used for assays based on SYBR Green dye as the detector and melt curve analysis (45–95°C) was performed after amplification.

Primer sequences and concentrations for each assay are summarized in Table 1. DNA from *Anaplasma* and *Ehrlichia* spp. was detected with a previously described SYBR assay, with a sensitivity of 10 gene copies, that targets the 16S rRNA gene.³⁴ *Bartonella* spp. were detected using a newly designed SYBR assay that targets the citrate synthase gene (Table 1); the assay has a sensitivity of 10 gene copies using *B. henselae* DNA and detects diverse *Bartonella* spp., including *B. bacilliformis*, *B. elizabethae*, *B. henselae*, *B. koehlerae*, *B. shoenbuchensis*, and *B. quintana*. The multicopy IS1111 transposable element of *Coxiella burnetii* was detected using a TaqMan assay with a lower limit of detection of one *C. burnetii* organism (Table 1). The 17-kd antigen gene of *Rickettsia* spp. was detected using previously described primers R17D135F and R17D249R,³⁵ with a newly designed probe (Table 1). The new probe, R17K-C, improved the sensitivity of the assay to 10 gene copies, and quantitation was accomplished using a 10-fold dilution curve of a plasmid containing the 17-kd antigen gene from *R. prowazekii*. These four assays use the same thermocycler conditions: 95°C for 10 minutes, followed by 40 cycles at 95°C for 15 seconds and 60°C for 60 seconds. The multicopy *pla* gene of *Yersinia pestis* was detected using a previously described SYBR Green assay and thermocycler conditions.³⁶

Conventional PCR and RFLP. Individual DNA samples that were detected by real-time PCR assays were used as the template for conventional PCR assays that amplify longer gene fragments. DNA from *Bartonella* spp. were amplified using primers that detect the 16S-23S ITS region (QHEV1/QHEV4) and the *groEL* gene (HSP1/BbHS1630.n).^{37,38} The superoxide dismutase gene of *C. burnetii* was amplified with a direct PCR assay.³⁹ DNA from *Rickettsia* was detected using PCR primers that amplify a 394-bp fragment of the 17-kd antigen gene (17kD-F1/17kD-R1).⁴⁰ If a product was not visible using agarose gel electrophoresis, the product of the conventional 17-kd PCR reaction was nested for 30 additional cycles using SFGR specific primers TZ15 and TZ16⁴¹ or using the real-time forward primer (R17K135F) with the conventional reverse primer (17kD-R1). In addition, the citrate synthase gene of *Rickettsia* was amplified from *E. gallinacea*,

TABLE 1
PCR primers and fluorescent probes used for real-time PCR testing of Egyptian fleas collected July 2002 to July 2003

Assay	Primer name	Sequence of primer or probe (5' to 3')										Concentration	
<i>Anaplasma/Ehrlichia</i> (SYBR)	EchSYBR-F	AAC	ACA	TGC	AAG	TCG	AAC	GG					75 nmol/L
	EchSYBR-R	CCC	CCG	CAG	GGA	TTA	TAC	A					75 nmol/L
<i>Bartonella</i> (SYBR)	BARH-CIT-F	TGC	TTC	GAC	ATC	CAC	TGT	ACG	TC				600 nmol/L
	BARH-CIT-R	CAC	CTG	CTG	CAA	TAC	ATG	CAA	ATG				800 nmol/L
<i>Coxiella burnetii</i> (TaqMan)	IS1111F	CCG	ATC	ATT	TGG	GCG	CT						1600 nmol/L
	IS1111R	CGG	CGG	TGT	TTA	GGC							800 nmol/L
	IS1111P*	TTA	ACA	CGC	CAA	GAA	ACG	TAT	CGC	TGT	G		200 nmol/L
<i>Rickettsia</i> (TaqMan)	R17K135F	ATG	AAT	AAA	CAA	GGK	ACN	GGH	ACA	C			800 nmol/L
	R17K249R	AAG	TAA	TGC	RCC	TAC	ACC	TAC	TC				800 nmol/L
	R17K-C*	TTG	GTT	CTC	AAT	TCG	GTA	AGG	GTA	AAG	G		100 nmol/L
<i>Yersinia pestis</i> (SYBR)	YP-PlaF	GTA	ATA	GGT	TAT	AAC	CAG	CGC	TT				500 nmol/L
	YP-PlaR	AGA	CTT	TGG	CAT	TAG	GTG	TG					500 nmol/L

* Fluorescent oligonucleotide probes were labeled with 5' FAM and a 3' fluorescence quencher (BHQ).

using primers RCPS877F and RCPS1258R.⁴² A PCR assay that amplifies the *pla* gene of *Y. pestis* was used as described by Stevenson and others⁴³ with the following modification: if a sample tested negative after 40 cycles of amplification, the assay was nested, using the same primers, for an additional 30 cycles of amplification. Direct and nested PCR amplicons were resolved by 2% agarose gel electrophoresis and visualized with ethidium bromide. Amplicons produced by the 17-kd antigenic gene PCR for *Rickettsia* were further characterized using restriction fragment length polymorphism (RFLP) analysis.⁴⁴ Amplicons were digested with *AluI* (New England BioLabs, Beverly, MA) for 6 hours at 37°C and resolved by 3% agarose gel electrophoresis.

Sequencing and GenBank accession numbers. PCR amplicons selected for DNA sequence analysis were prepared using the QIAquick PCR Purification Kit (Qiagen, Valencia, CA). Sequencing reactions were performed in duplicate, using the forward and reverse PCR primers and the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems). Excess dye was removed using DyeEx 2.0 columns (Qiagen). Sequences were resolved with the ABI 3100 capillary sequencer (Applied Biosystems). Sequence fragments were aligned with SeqMerge (Accelrys, San Diego, CA), and primer sequences were removed. The resulting sequences were compared with published sequences in GenBank using Blast 2.0.⁴⁵ Representative sequences were submitted to GenBank, as follows: *Bartonella* spp. *groEL* gene PCR amplicons from *X. cheopis*, “A” DQ166941 and “B” DQ166942; *Bartonella* sp. “C” ITS amplicon from *X. cheopis* and *L. segnis*, DQ166943; *Bartonella* sp. “D” ITS amplicon from *X. cheopis*, DQ166944; *Bartonella* sp. “E” *groEL* gene PCR amplicon from *L. segnis*, DQ166945; *Coxiella* sp. superoxide dismutase gene PCR amplicon from *X. cheopis*, DQ166935; *Rickettsia typhi* 17-kd gene amplicon, DQ166936; *Rickettsia* sp. 17-kd gene amplicon from *E. gallinacea*, DQ166937; *Rickettsia* sp. citrate synthase gene amplicon from *E. gallinacea*, DQ166938; and *Rickettsia* spp. 17-kd antigenic gene sequences from *X. cheopis*, DQ166939 and DQ166940.

Phylogenetic analysis. Phylogenetic analysis was used to compare the DNA sequences from *groEL* gene amplicons from Egypt with published sequences from *B. alsatica* (AF299357), *B. birtlesii* (AF355773), *B. bacilliformis* (M98257), *B. clarridgeiae* (AF014831), *B. doshiae* (AF014832), *B. elizabethae* (AF014834), *B. grahamii* (AF014833), *B. henselae* (AF304020), “*Bartonella phoceensis*” (AY515129), “*Bartonella rattimassiliensis*” (AY515128), *B. quintana* (AF014830), *B. taylorii* (AF304017), and *B. vinsonii berkhoffii* (AF014836). Sequences were aligned using ClustalW.⁴⁶ Unrooted parsimony analysis (1,002 bp) of the aligned sequences was performed using the Phylip 3.62 software package,⁴⁷ and 100 bootstrap replicates were performed.

Statistics. χ^2 analysis was used to compare the proportion of male and female fleas infected with of *R. typhi*, the proportion of *R. typhi*-infected fleas collected from black rats versus Norway rats, and the proportion of *R. typhi*-infected fleas at each individual site.

RESULTS

Flea collection. Between July 2002 and July 2003, a total of 247 animals from 17 cities throughout Egypt were collected and examined for fleas. Fleas were collected from 224 animals

(90.6% infestation rate). A representative sample of fleas was slide-mounted for identification, and the remaining 987 fleas from 221 animals were tested for DNA from bacterial agents (Table 2). Nine hundred (91.2%) of these fleas were *X. cheopis*, including 459 females and 441 males, and all but 1 were collected from either *Rattus norvegicus* (Norway rat, $N = 466$), or *Rattus rattus* (black rat, $N = 433$). The remaining fleas included 38 *C. felis* (22 females, 16 males), 37 *L. segnis* (19 females, 18 males), and 12 female *E. gallinacea*. These fleas were collected from rats, a domestic goat (*Capra hircus*), a house mouse (*Mus musculus*), three least weasels (*Mustela nivalis*), and four Rueppel’s foxes (*Vulpes rueppelli*).

Anaplasma and Ehrlichia. Fleas were tested for *Anaplasma* and *Ehrlichia* DNA using a SYBR Green real-time PCR assay that detects *A. phagocytophilum*, *E. canis*, *E. chaffeensis*, *E. ewingii*, and *E. muris*, with a sensitivity of 10 gene copies per microliter of template DNA, which corresponds to ~500–600 genomes per flea.^{34,48} All 328 pooled DNA samples, representing 987 individual fleas, were negative using this assay.

Bartonella. Using a real-time PCR assay, DNA from *Bartonella* spp. was detected in 21 fleas, including 1 *L. segnis* and 20 *X. cheopis*, from Alexandria, Mansoura, and Mokattam Village (Cairo). Eleven of these fleas were positive using a conventional PCR assay for the 16S-23S ITS region of *Bartonella*, and 17 were positive using a PCR assay for the *Bartonella groEL* gene. Two distinct *groEL* sequences and two distinct ITS sequences were obtained from these fleas (Table 3). A short fragment of the *groEL* amplicon from *L. segnis* was sequenced using the reverse PCR primer and seems to represent a third *groEL* genotype (“E”), but all attempts to sequence the amplicon using the forward primer yielded mixed sequences, suggesting coinfection of this flea with an unidentified *Bartonella* sp. According to a BLAST analysis performed using these sequences, all five sequences represent previously unreported *Bartonella* spp. The *groEL* “A” sequence was 89% similar to *B. alsatica* and “*B. phoceensis*”; *groEL* “B” was 92% similar to *B. clarridgeiae*; *groEL* “E” was 95% similar to *B. tribocorum* and 92% similar to *B. elizabethae*; ITS “C” was 90–93% similar to unnamed *Bartonella* spp. from *C. felis* (strain CtF4YN, AY566176) and *Rattus tanezumi flavipectus* from China (strain Rt222sm, AY277896); and ITS “D” was 85–90% similar to *B. tribocorum*, *B. grahamii*, and *B. elizabethae*. Unrooted parsimony analysis of 1,002 bp of the *groEL* gene sequence showed strong support that genotype “B” is related to *B. clarridgeiae* (100/100 bootstrap replicates), and genotype “A” formed a clade with *B. alsatica* and “*B. phoceensis*” (Figure 1).

Coxiella. Using real-time PCR, *Coxiella burnetii* was detected in two fleas: one *C. felis* from a weasel trapped in Zagazig (C_T 34.0, ~50–60 organisms/flea) and one *X. cheopis* from a Norway rat collected in Alexandria (C_T 29.9, ~800–1,000 organisms/flea). PCR amplification of the superoxide dismutase gene was successful with the *X. cheopis* DNA extract, and the sequence of the PCR amplicon was 98% homologous (210/214 bp) to *Coxiella burnetii* (M74242).

Rickettsia. DNA of *Rickettsia* was detected using a real-time PCR assay that amplifies and quantifies the 17-kd antigen gene of all known SFGR and TGR spp. Using this assay, 41 individual fleas were positive for *Rickettsia* spp.: 12/12 (100%) *E. gallinacea*, from five black rats trapped in Mansoura and Zagazig, 1/36 (2.8%) *L. segnis*, collected from a black rat from Mansoura, and 28/900 (3.1%) *X. cheopis*. Am-

TABLE 2
Flea collection summary from 17 cities in Egypt, 2002–2003

Collection location	Date of collection	Host animals	<i>n</i>	Fleas collected	<i>n</i>	No. female/no. male
Alexandria	12–14 Nov. 2002	<i>Rattus norvegicus</i>	29	<i>C. felis</i>	2	1/1
				<i>X. cheopis</i>	173	106/67
Aswan	21 April 2003	<i>R. norvegicus</i>	1	<i>X. cheopis</i>	8	5/3
El Kharga Oasis	15 March 2003	<i>Rattus rattus</i>	5	<i>X. cheopis</i>	27	12/15
Fayoum	16–19 June 2003	<i>R. norvegicus</i>	2	<i>X. cheopis</i>	8	0/8
		<i>R. rattus</i>	6	<i>X. cheopis</i>	14	6/8
		<i>Mustela nivalis</i>	1	<i>C. felis</i>	3	1/2
Mokattam Village (Cairo)	7–8 Aug. 2002	<i>R. norvegicus</i>	25	<i>C. felis</i>	1	1/0
				<i>X. cheopis</i>	131	53/78
		<i>R. rattus</i>	2	<i>X. cheopis</i>	19	10/9
Hurghada	2–3 Feb. 2003	<i>Mus musculus</i>	1	<i>X. cheopis</i>	1	1/0
		<i>R. norvegicus</i>	3	<i>X. cheopis</i>	1	5/3
		<i>R. rattus</i>	10	<i>X. cheopis</i>	23	13/10
Ismailia	29 Aug. 2002	<i>R. norvegicus</i>	7	<i>X. cheopis</i>	29	11/18
Mansoura	2–3 Dec. 2002	<i>R. rattus</i>	31	<i>E. gallinacea</i>	3	3/0
				<i>L. segnis</i>	26	13/13
				<i>X. cheopis</i>	161	86/75
Matrouh	18–19 Dec. 2002	<i>R. rattus</i>	2	<i>L. segnis</i>	2	2/0
				<i>X. cheopis</i>	2	0/2
Port Said	26–27 Nov. 2002	<i>R. norvegicus</i>	8	<i>C. felis</i>	1	1/0
				<i>X. cheopis</i>	16	6/10
		<i>R. rattus</i>	8	<i>L. segnis</i>	1	1/0
				<i>X. cheopis</i>	11	3/8
Qara	24–25 Jan. 2003	<i>R. rattus</i>	10	<i>C. felis</i>	6	3/3
				<i>X. cheopis</i>	57	33/24
		<i>Vulpes rueppelli</i>	4	<i>C. felis</i>	11	8/3
Quseir	20 March 2003	<i>R. norvegicus</i>	12	<i>X. cheopis</i>	64	29/35
Safaga	4–5 Feb. 2003	<i>R. norvegicus</i>	7	<i>X. cheopis</i>	20	12/8
		<i>R. rattus</i>	2	<i>X. cheopis</i>	11	5/6
Siwa Oasis	23–25 July 2002	<i>R. rattus</i>	2	<i>X. cheopis</i>	3	2/1
St. Catherine	14–17 July 2003	<i>Capra hircus</i>	1	<i>C. felis</i>	3	2/1
Suez	21–23 Oct. 2002	<i>R. rattus</i>	17	<i>C. felis</i>	1	1/0
				<i>L. segnis</i>	3	2/1
				<i>X. cheopis</i>	48	24/24
Wadi El Natroun	11–14 Aug. 2002	<i>R. norvegicus</i>	4	<i>X. cheopis</i>	9	6/3
		<i>R. rattus</i>	8	<i>X. cheopis</i>	35	17/18
Zagazig	3–31 Oct. 2002	<i>R. rattus</i>	11	<i>E. gallinacea</i>	9	9/0
				<i>L. segnis</i>	5	1/4
				<i>X. cheopis</i>	22	14/8
		<i>M. nivalis</i>	2	<i>C. felis</i>	10	4/6

plification of a larger fragment of the same gene, using conventional PCR primers,⁴⁰ was successful for all 12 *E. gallinacea*, the 1 *L. segnis*, and 16 of the 28 *X. cheopis* that were positive using the real-time PCR assay. An additional 11 *X. cheopis* produced amplicons using nested PCR. The one *X. cheopis* DNA extract that was negative using the nested PCR assay contained only 26.4 ± 0.3 copies of this gene per microliter. RFLP analysis of the conventional, non-nested PCR amplicons with *AluI* produced two patterns: amplicons from *X. cheopis* and *L. segnis* produced a doublet at ~220 and 200 bp, and amplicons from *E. gallinacea* produced distinct bands at ~190 and 120 bp (data not shown).

Sequence analysis was performed on all conventional PCR amplicons from *L. segnis*, *X. cheopis*, and *E. gallinacea*. The sequences of the 17-kd amplicons from 25 *X. cheopis* and the *L. segnis* were 100% similar to each other and to published sequences for *R. typhi*, with predicted *AluI* RFLP fragments at 228 and 196 bp. Two of the *X. cheopis* contained DNA from SFGR (26–118 copies of the 17-kd gene/ μL DNA, ~1,300–7,080 genomes per flea); the sequence of the amplicon from one *X. cheopis* from a black rat in Suez was 100% (116/116 bp) similar to “*R. amblyommii*”, *R. cooperi*, *R. honei*, *R. parkeri*, and *R. rickettsii*; and the amplicon from a *X. cheo-*

pis from a Norway rat in Alexandria was 97% similar (113/116 bp) to *R. conorii* and *R. montanensis*. The small size of the nested PCR fragments precluded definitive identification of these agents to the species level.

The 25 *X. cheopis* containing DNA from *R. typhi* are listed in Table 4 and were collected from Norway rats (13 fleas) and black rats (12 fleas) from 9 of the 17 sampled cities: Alexandria, Mokattam Village (Cairo), Ismailia, Mansoura, Port Said, Quseir, Safaga, Suez, and Wadi El Natroun. The number of 17-kd gene copies in these fleas varied from < 100 to $> 5 \times 10^5/\mu\text{L}$ of DNA extract. Both male (11 fleas) and female (14 fleas) fleas contained DNA from *R. typhi*. There was no statistically significant difference in the prevalence of *R. typhi* infection between male and female fleas ($P = 0.598$) or between fleas collected from black rats and Norway rats ($P = 0.986$). The proportion of fleas containing *R. typhi* was significantly higher in fleas collected from Suez (12.5%, 6/48, $P = 0.0002$); however, five of these six fleas containing *R. typhi* DNA came from the same host animal.

The number of 17-kd antigen gene copies in each microliter of DNA from *E. gallinacea* ranged from 5,286 to 86,525 (mean, 21,732 copies/ μL). The sequences of the 17-kd amplicons from *E. gallinacea* were 100% homologous to each other

TABLE 3

Detection of *Bartonella* spp. DNA in 21 Egyptian fleas collected July 2002 to July 2003: individual flea collection details and *Bartonella* gene sequences

Collection location	Host species	Host ID*	Species of flea	Flea sex	groEL sequence	ITS sequence
Alexandria	<i>R. norvegicus</i>	209	<i>X. cheopis</i>	F	groEL "A"	No amplification
	<i>R. norvegicus</i>	220	<i>X. cheopis</i>	M	groEL "A"	No amplification
	<i>R. norvegicus</i>	221	<i>X. cheopis</i>	F	groEL "A"	ITS "C"
	<i>R. norvegicus</i>	223	<i>X. cheopis</i>	F	groEL "B"	ITS "C"
	<i>R. norvegicus</i>	229	<i>X. cheopis</i>	F	groEL "B"	No amplification
Mansoura	<i>R. rattus</i>	306	<i>X. cheopis</i>	F	groEL "A"	No amplification
	<i>R. rattus</i>	320	<i>X. cheopis</i>	F	groEL "A"	No amplification
	<i>R. rattus</i>	326	<i>L. segnis</i>	F	Mixed; groEL "E"	ITS "C"
	<i>R. rattus</i>	327	<i>X. cheopis</i>	F	No amplification	ITS "C"
	<i>R. rattus</i>	327	<i>X. cheopis</i>	M	groEL "A"	No amplification
	<i>R. rattus</i>	327	<i>X. cheopis</i>	M	groEL "A"	No amplification
	<i>R. rattus</i>	335	<i>X. cheopis</i>	F	groEL "A"	ITS "C"
	<i>R. rattus</i>	337	<i>X. cheopis</i>	M	groEL "A"	No amplification
	<i>R. norvegicus</i>	41	<i>X. cheopis</i>	M	No amplification	ITS "C"
Mokattam Village (Cairo)	<i>R. rattus</i>	44	<i>X. cheopis</i>	F	groEL "A"	No amplification
	<i>R. rattus</i>	44	<i>X. cheopis</i>	M	groEL "A"	No amplification
	<i>R. norvegicus</i>	66	<i>X. cheopis</i>	M	No amplification	ITS "C"
	<i>R. norvegicus</i>	67	<i>X. cheopis</i>	F	No amplification	ITS "D"
	<i>R. norvegicus</i>	67	<i>X. cheopis</i>	F	groEL "A"	ITS "C"
	<i>R. norvegicus</i>	69	<i>X. cheopis</i>	F	groEL "B"	Mixed sequence
	<i>R. norvegicus</i>	73	<i>X. cheopis</i>	M	groEL "A"	ITS "C"

* Host ID is the unique identification number assigned to each animal collected during the course of the study.

and to an unnamed *Rickettsia* from *C. felis* collected in South Carolina (AY953286), but only 96% homologous to the closest named *Rickettsia* spp.: *R. australis* (M74042, 381/394 bp similarity) and *R. felis* (AF195118, 379/394 bp). Predicted RFLP fragments after digestion of this 17-kd amplicon with *AluI* were 184, 129, and 109 bp in size. The unnamed *Rickettsia* from *E. gallinacea* was further characterized by amplification and sequencing of 341 bp of the citrate synthase gene. The sequence was 100% homologous to sequences from an unnamed *Rickettsia* sp. from *Ctenocephalides* spp. in South Carolina (AY953289) and Thailand (RF2125 genotype, AF516333).^{49,50}

***Yersinia pestis*.** The *pla* gene of *Y. pestis* was not detected in any of the 987 DNA extracts derived from Egyptian fleas. Equivocal real-time PCR results were obtained for seven pools of DNA, in which small peaks were seen after 40–45 cycles of amplification, possibly representing trace quantities of the target gene or non-specific amplification of DNA. All 21 individual fleas from these pools were negative when evaluated using a conventional, nested PCR assay.

DISCUSSION

Fleas were collected from small- and medium-sized mammals at 17 cities distributed throughout Egypt. Although four species of fleas were represented in the collection, the majority were *X. cheopis* (91.4%), with smaller numbers of *C. felis*, *E. gallinacea*, and *L. segnis*. This is consistent with previously described collections of ectoparasites from rodents and foxes in Egypt.^{16,19,24–26}

Previously undescribed *Bartonella* spp. were detected in several *X. cheopis* and one *L. segnis* collected in Egypt. The conventional PCR amplicons obtained from these fleas produced three sequences for the *groEL* gene and two for the 16S–23S ITS region. All of these sequences represent undescribed *Bartonella* genotypes. Phylogenetic analysis of the *groEL* gene sequences suggests that one of these new *Bar-*

tonella sp. is related to *B. clarridgeiae* and might, therefore, be pathogenic to humans. Further studies are needed to assess the pathogenicity of the novel *Bartonella* spp., their antigenic similarity to pathogens of humans or domestic animals, and the exposure of humans in Egypt to flea-borne *Bartonella* infections.

Coxiella burnetii was detected in 2 of the 987 fleas, and definitive identification was possible in a *X. cheopis* from Alexandria. This low infection rate is not unexpected, because *C. burnetii* is transmitted primarily by aerosol, milk

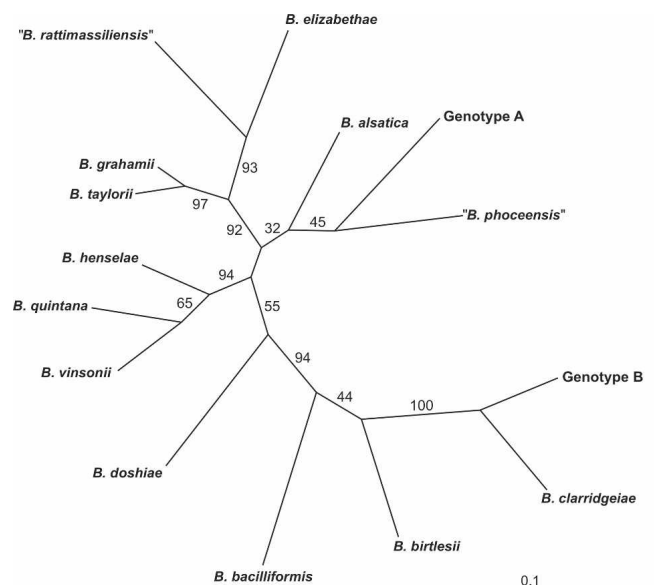


FIGURE 1. Unrooted parsimony tree showing the relationship between the *groEL* gene sequences (1,002 bp) of genotypes A and B from Egyptian fleas and sequences from previously described *Bartonella* spp. The scale bar indicates the number of changes per 100 bp, and bootstrap values (of 100 replicates) are indicated on individual branches.

TABLE 4

Flea collection details and comparison of real-time PCR and conventional PCR assays for 28 *X. cheopis* containing DNA from *Rickettsia* spp.

Collection location	Host species	Host ID*	Flea sex	Copies/ μ L DNA†	Conventional PCR	<i>Rickettsia</i> species
Alexandria	<i>R. norvegicus</i>	221	F	167	+	<i>R. typhi</i>
	<i>R. norvegicus</i>	226	F	109	+	<i>R. typhi</i>
	<i>R. norvegicus</i>	226	M	54	+	<i>R. typhi</i>
	<i>R. norvegicus</i>	227	F	76	+	<i>R. typhi</i>
	<i>R. norvegicus</i>	227	F	118	+	SFGR
	<i>R. norvegicus</i>	233	F	37	+	<i>R. typhi</i>
Ismailia	<i>R. norvegicus</i>	237	F	26	-	ND
	<i>R. norvegicus</i>	115	F	93	+	<i>R. typhi</i>
Mansoura	<i>R. rattus</i>	328	F	326	+	<i>R. typhi</i>
	<i>R. rattus</i>	328	F	13	+	<i>R. typhi</i>
	<i>R. rattus</i>	328	M	52,280	+	<i>R. typhi</i>
	<i>R. rattus</i>	335	M	18,742	+	<i>R. typhi</i>
Mokattam Village (Cairo)	<i>R. norvegicus</i>	65	F	264	+	<i>R. typhi</i>
	<i>R. norvegicus</i>	73	F	536,435	+	<i>R. typhi</i>
	<i>R. norvegicus</i>	73	M	1,848	+	<i>R. typhi</i>
	<i>R. norvegicus</i>	76	F	616	+	<i>R. typhi</i>
Port Said	<i>R. norvegicus</i>	277	F	18,361	+	<i>R. typhi</i>
	<i>R. rattus</i>	281	M	60	+	<i>R. typhi</i>
Quseir	<i>R. norvegicus</i>	521	M	173	+	<i>R. typhi</i>
Safaga	<i>R. norvegicus</i>	461	M	311	+	<i>R. typhi</i>
Suez	<i>R. rattus</i>	149	F	26	+	SFGR
	<i>R. rattus</i>	158	F	47,070	+	<i>R. typhi</i>
	<i>R. rattus</i>	158	M	88,673	+	<i>R. typhi</i>
	<i>R. rattus</i>	158	F	64	+	<i>R. typhi</i>
	<i>R. rattus</i>	158	F	301	+	<i>R. typhi</i>
	<i>R. rattus</i>	158	M	282	+	<i>R. typhi</i>
	<i>R. rattus</i>	164	M	4,262	+	<i>R. typhi</i>
	<i>R. rattus</i>	83	M	178	+	<i>R. typhi</i>

* Host ID is the unique identification number assigned to each animal collected during the course of the study.

† Quantitative real-time PCR was used to determine the mean number of copies of the 17 kd antigen gene per microliter of flea DNA extract.

ND, not determined.

products, or ticks.⁵² Similarly, we did not detect *Anaplasma* and *Ehrlichia* spp. in fleas from Egypt; these agents are transmitted by ticks but have not been described from fleas.⁵³⁻⁵⁵

Rickettsia typhi, the agent of murine typhus, was detected in 25 (2.8%) of the *X. cheopis* and in one (2.7%) *L. segnis*, and SFGR were detected in 2 (0.2%) of the *X. cheopis*. Typhus-positive fleas were collected from both Norway and black rats from nine cities in the Nile Delta, Suez Canal area, and the coast of the Red Sea. We did not detect DNA from *R. typhi* from fleas from eight other cities in Egypt, but this finding was not statistically significant because of the small number of *X. cheopis* collected from these areas. These data are, however, consistent with serosurveys from Egypt that have recorded a higher prevalence of antibodies against TGR versus SFGR and that have documented the presence of antibodies versus TGR in the Nile River Delta.^{8,10,15,56} The number of rickettsial 17-kd antigen gene copies in the fleas containing *R. typhi* DNA ranged from $10^{1.1}$ to $10^{5.7}$ per microliter of DNA, and the average volume of DNA from each flea was 50–60 μ L; therefore, the number of gene copies per flea was $\sim 10^3$ – 10^8 . Similar titers of *R. typhi* have been reported 7–32 days after experimental infection of *X. cheopis*,⁵⁷ and these data suggest that the *X. cheopis* collected in Egypt were infected with the pathogen at the time of collection. The number of *R. typhi* gene copies detected in *L. segnis* was near the low end of this range ($10^{3.3}$ – $10^{3.6}$) and could represent either infection of the flea or residual DNA from a blood meal. These data document the presence of *R. typhi* in an enzootic *Rattus/X. cheopis* cycle in Egypt, and this enzootic cycle provides a reservoir for continued human exposure to TGR.

The SFGR in *X. cheopis* could not be identified conclu-

sively to the species level, because of the low number of copies and the short fragment obtained by nested PCR. The sequences of the short amplicons produced by the nested PCR assay suggest that one of the fleas contained DNA from *R. conorii* (or a similar agent) and the other contained DNA from an unnamed SFGR. Detection of DNA from SFGR in *X. cheopis* could reflect residual DNA acquired during a blood meal or infection of the fleas.

Only 12 *E. gallinacea* were collected, from five black rats in the Nile Delta, but all 12 fleas contained DNA from an unnamed *Rickettsia* sp. This high infection rate (100%) and large number of gene copies detected in these fleas might be because of efficient vertical transmission of the unknown agent, as has been described for *Rickettsia felis* in *C. felis*⁵⁸ or because of horizontal transmission. *Echidnophaga gallinacea* has been collected from rodents, dogs, and foxes in Egypt^{59,60} and has been reported to bite humans.⁶¹ It is possible, therefore, that humans or domestic animals could be exposed to this agent. Similar, possibly identical, *Rickettsia* have been described from *Ctenocephalides* spp. in the United States and Thailand, but the pathogenicity of this agent has not been determined.^{49,50} Additional studies, using a larger sample of *E. gallinacea* from Egypt, are needed to confirm the high infection rate in this flea, to determine whether humans or animals are, in fact, exposed to the rickettsial agent, and to establish the pathogenicity of the agent.

It is noteworthy, given the historical reports of plague epidemics in Egypt^{1,2} and the fact that *X. cheopis* is considered to be the primary vector of this agent, that *Y. pestis* was not detected in any of the 900 *X. cheopis* we sampled. These data are compatible with the reported disappearance of plague

from Egypt in the second half of the 20th century² and with the absence of reported plague cases from Egypt in recent times.

In summary, we identified DNA from *Bartonella* spp., *Coxiella burnetii*, *Rickettsia typhi*, and an unnamed SFGR agent in fleas collected from Egypt between July 2002 and July 2003. Two additional SFGR were identified in fleas but definitive species identification was not possible. *R. typhi* and *C. burnetii* are known to be pathogenic to humans, and human exposure to these agents has been documented multiple times during the last century. The medical and veterinary significance of the previously undescribed *Bartonella* spp. and the unnamed SFGR genotype remain to be determined; these agents may be benign symbionts of the fleas, rodent pathogens ingested during a blood meal, or they may be emergent pathogens of public health significance.

Received September 12, 2005. Accepted for publication February 21, 2006.

Acknowledgments: The authors thank Maria Badra, Alaa Taher, Emad El Din Yehia, and Ahmed Fawzi for invaluable support provided in Egypt, and Herbert Thompson and Rachel Priestley, Centers for Disease Control and Prevention, Atlanta, GA, for permitting us to use a previously unpublished real-time PCR assay for *Coxiella burnetii*. Special thanks are extended to the team members from the Vector Biology Department at the Egyptian Ministry of Health for their great support in the field work for this study.

Financial support: This work was supported by GEIS, Work Unit 847705.82000.25GB.E0018. The opinions and assertions contained herein are the private views of the authors and are not to be construed as official or reflecting the views of the Department of the Navy, Department of Defense, Department of Health and Human Services, or the United States Government.

Authors' addresses: Amanda D. Loftis, Will K. Reeves, John R. Moriarity, and Gregory A. Dasch, CDC, 1600 Clifton Road NE, MS G-13, Atlanta, GA 30333, E-mails: aloftis@cdc.gov, WReeves1@cdc.gov, JMoriarity@cdc.gov, and GDasch@cdc.gov. Daniel E. Szumlas, Navy Disease Vector Ecology and Control Center, Box 43, NAS, Jacksonville, FL, 32212-0043, E-mail: SzumlasD@namru3.med.navy.mil. Magda M. Abbassy and Ibrahim M. Helmy, United States Naval Medical Research Unit No. 3, PSC 452, Box 5000, FPO AE 09835-0007.

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