SURVEILLANCE OF EGYPTIAN FLEAS FOR AGENTS OF PUBLIC HEALTH SIGNIFICANCE: ANAPLASMA, BARTONELLA, COXIELLA, EHRLICHA, RICKETTSSIA, AND YERSINIA PESTIS

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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 1946.5–7 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 rattus8,10 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 peri-domestic, urban mammals from 17 cities in Egypt.

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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) and Osborn and Helmy (1980). 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) and Hooistraald and Traub (1965). 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, 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). 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.

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Prime 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. 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. shoebuchensis, 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, 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.

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). The superoxide dismutase gene of C. burnetii was amplified with a direct PCR assay. DNA from Rickettsia was detected using PCR primers that amplify a 394-bp fragment of the 17-kd antigen gene (17KD-F1/17KD-R1). 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 TZ16 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,
using primers RCPS877F and RCPS1258R. A PCR assay that amplifies thepla gene of Y. pestis was used as described by Stevenson and others 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. 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. 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; Coxella 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 sp. 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. claridgeiae (AF014831), B. dushiae (AF014832), B. elizabethae (AF014834), B. grahamii (AF014833), B. henselae (AF304020), Bartonella phoebeensis (AY15129), Bartonella ratti nassiliensis (AY15128), B. quintana (AF014830), B. taylorii (AF304017), and B. vinsonii berkofffii (AF014836). Sequences were aligned using ClustalW. Unrooted parsimony analysis (1,002 bp) of the aligned sequences was performed using the Phylib 3.62 software package, and 100 bootstrap replicates were performed.

**Statistics.** χ² analysis was used to compare the proportion of male and female fleas infected with 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 Rueppell’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. All 382 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. phoebeensis”; groEL “B” was 92% similar to B. claridgeiae; 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 CtF4Y, AY566176) and Rattus tanezumi flaviceps from China (strain R222sm, 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. claridgeiae (100/100 bootstrap replicates), and genotype “A” formed a clade with B. alsatica and “B. phoebeensis” (Figure 1).

**Coxella.** Using real-time PCR, Coxella burnetii was detected in two fleas: one C. felis from a weasel trapped in Zaqazag (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 Coxella burnetii (M74242).

**Rickettsia.** DNA of Rickettsia was detected using a real-time PCR assay that amplifies and quantifies the 17-kd antigenic 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-
amplification of a larger fragment of the same gene, using conventional PCR primers, 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 *Alu*I 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 *Alu*I RFLP fragments at 228 and 196 bp. Two of the *X. cheopis* contained DNA from SFGR (26,4) similar to *L. segnis*. The proportion of fleas containing *R. typhi* infection between male and female fleas (\( P = 0.098 \)) 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 micro-liter 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.
Detection of Bartonella spp. DNA in 21 Egyptian fleas collected July 2002 to July 2003: individual flea collection details and Bartonella gene sequences

<table>
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<th>Collection location</th>
<th>Host species</th>
<th>Host ID*</th>
<th>Species of flea</th>
<th>Flea sex</th>
<th>groEL sequence</th>
<th>ITS sequence</th>
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* 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 *Bartonella* 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
products, or ticks. 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.

*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 Norwegian 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. The number of rickettsial 17-kd antigen gene copies in the fleas containing *R. typhi* DNA ranged from 10^1.3^ to 10^1.7^ per microliter of DNA, and the average volume of DNA from each flea was 50–60 μL; therefore, the number of gene copies per flea was ~10^3–10^5. Similar titers of *R. typhi* have been reported 7–32 days after experimental infection of *X. cheopis* 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 (10^1.3^–10^1.6^) 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 conclusively 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 flea.

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* and because of horizontal transmission. *Echidnophaga gallinacea* has been collected from rodents, dogs, and foxes in Egypt and has been reported to bite humans. 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. 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 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 century 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.

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