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# Plague entomological and rodent surveillance in West and Northeast of Iran

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**Abstract**
The present study was performed to assess the infection of rodent reservoirs and their fleas to plaque agent (*Yersinia pestis*) in Kurdistan and North Khorasan respectively as active endemic and inactive non-endemic foci in the country. Live traps were used to collect rodents in spring and summer 2015. Totally, 109 rodents were trapped. Rodents of genus *Apodemus witherbyi* (40.5%) were the most abundant species in Kurdistan whereas *Meriones persicus* was the only species found in North Khorasan. *Meriones* spp constituted 12% of the trapped rodents in Kurdistan. 105 fleas were collected, of which 53(50.5%) were *Xenopsylla buxtoni*. The results of assays of rodent and flea specimens were negative for plague agent. Since rodents of *Meriones* spp and *Apodemus* spp and fleas of *Xenopsylla* spp in turn are the known reservoirs and vectors for plague in Iran, there is a real potential for the reemerging of plague disease in the studied areas.

**Keywords:** *Yersinia pestis*, plague, rodent, flea, Iran

## 1. Introduction
Plague is a fatal infectious disease that is endemic in various regions around the world, including Iran [1–3]. Clinical forms of the disease including bubonic, pneumonic, septicemic and pharyngeal may occur in humans [4]. The case fatality rate may reach to 100% if patient left without rapid and effective treatment, particularly for pneumonic plague [3]. The disease caused by the bacillus bacterium of *Yersinia pestis*, which is naturally circulated among wild rodents via infected flea bites particularly the *Xenopsylla* genus [5–7].
In Iran, several plague outbreaks have been recorded during 19th and 20th centuries including the epidemics in 1772-1773, 1829, 1835, 1870, and 1946-1965, resulted in hundreds to millions human deaths [8–12]. Since 1947, several studies have shown that rodents were plague-infected or possibly infected to the bacillus that actively is circulated among wild rodents in western Iran particularly in Kurdistan [1, 9, 13, 14]. Literature has shown that four rodent species of *Meriones persicus, M. libycus, M. vinogradovi* and *M. tristrami* are important in natural cycle of the bacterium in western Iran. The first two species were resistant and the later ones were sensitive to plague. Moreover, *Tatera indica* is also considered as plague vector in Iran [15]. Fleas of *Xenopsylla buxtoni* are the main plague vector in Western Iran [9], however other flea species such as *Xenopsylla cheopis, X. astia, X. conformis, Nosopsyllus fasciatus, N. iranus iranus*, and *Stenoponia tripectinata* are also incriminated as *Y. pestis* vectors within and among vertebrates [11, 15, 16].
Kurdistan is known as one of the most important endemic focus canters of plague in Iran [9, 14, 16–18]. This region is located in west of the country and is stretched across southern Turkey and the north of Iraq and Syria. Several outbreaks have occurred in Kurdistan with numerous human deaths [8, 19, 20], the last one goes back to 1966-1967 which resulted in 156 human deaths [21]. A serological study in 2011- 2012 in the provincial border line between Kurdistan with Hamadan provinces showed that the rodent and dogs were antibody positive against the plague agent, indicating the region is still active [1].
Khorasan region in northeast of Iran is a very old plague focus, with a few outbreaks in 1829-1833, 1877 (37 deaths), 1913, and 1921 with unidentified human deaths [8–10, 16]. Khorasan, the largest province in Iran until 2004 civil-administrative changes, has been divided into three provinces of Razavi, South, and North Khorasan Provinces as a result. There has been no plague report from Khorasan since 1921.

Since the plague agent circulates at low levels in the wild rodent populations in the endemic foci, could persist active for several years in the burrows "burrowing plague" [21], the disease remains silent for decades shows a periods of silence and re-emergence, the reemergence and outbreaks are always possible [22, 23]. In addition, the reemergence of the plague in Iran is more likely since several plague outbreaks have been reported by WHO in the Eastern Mediterranean Region particularly in neighboring countries of Iran including Afghanistan, Jordan and Saudi Arabia in last 20 years [19]. These facts prompted us to study the rodent and flea diversity and plague infection in these two plague endemic foci with North Khorasan as a part of a very old non-active focus in northeast and Kurdistan as an active focus in the west of Iran to assess the possible plague reemergence.

## 2. Materials and Methods

### 2.1 Rodent and flea collection

Rodent collection was performed in 18 villages in Kurdistan and six villages in North Khorasan in spring and summer 2015 (Fig. 1 and Table 4). These villages are distributed in Marivan, Sanandaj, and Sarvabad districts from Kurdistan and Bojnord and Razojergelan from North Khorasan. The animals were trapped using traditional rodent live traps supplemented by baits including dates, walnut, and bread applied with vegetable oil, as well as cheese puffs. The Global Positioning System (GPS) was used to record details of rodent collection sites. To isolate the rodent ectoparasites particularly fleas, the trapped animals were held with a long forceps and kept over a bowl filled with water. Ectoparasites were released into the water by slowly brushing and blowing in the rodent fur. The fleas were collected from the water with forceps or brushes and placed individually into microtubes filled with 70% ethanol. The flea specimens were shipped to the Department of Medical Entomology and Vector Control, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran, for species identification. Then the trapped rodents were sprayed by pyrethroid insecticides and sent to the local research laboratories for morphological identification using identification key [24]. The rodents were anesthetized with chloroform and after taking some blood by heart puncture, they were killed. After dissection, a piece of spleen was aseptically removed and kept at -4 C. The spleen and blood specimens were sent to the Research Center for Emerging and Reemerging Diseases, the national Reference laboratory for Plague, Tularemia and Q fever, Pasteur Institute of Iran for further molecular studies. All procedures were performed in accordance with the terms of the Iran Animals (Scientific Procedures) Act Project License and were approved by the Tehran University of Medical Sciences Ethical Review Committee.

### 2.2 DNA extraction and Real-time PCR

Genomic DNA was extracted from each flea or spleen specimens using the Qiagen DNA Extraction Kit and subsequently stored at -70$^{0C}$ until use. Real-time PCR was performed using the Rotor-Gene 6600 (6-Plex) real time PCR System (Corbett Life Science) by the method already described by Stewart *et al* [25]. *Yersinia pestis*-positive controls were the chromosomal *yihN* gene and the pMT1-borne *caf1* gene cloned into plasmid pUC57 (provided by the Pasteur Institute of Iran). The Real time PCR assays were performed against three genes of 1) *yihN* that is a chromosomal gene shared between three species of *Y. pestis, Y. speudotuberculosis,* and *Y. enterocolitica*, 2) *caf1*(pMT1) and 3) *Pla*(pPCP1) that are two specific plasmid genes for *Y. pestis*. The assays were done using four pairs of primers and their corresponding probes (Table 1) using the following thermal program: initial denaturation at 95 for 10 mins followed by 45 cycles of denaturation at 95 for 15 seconds, annealing at 58 for 30 seconds, and extension at 72 for 30 seconds.

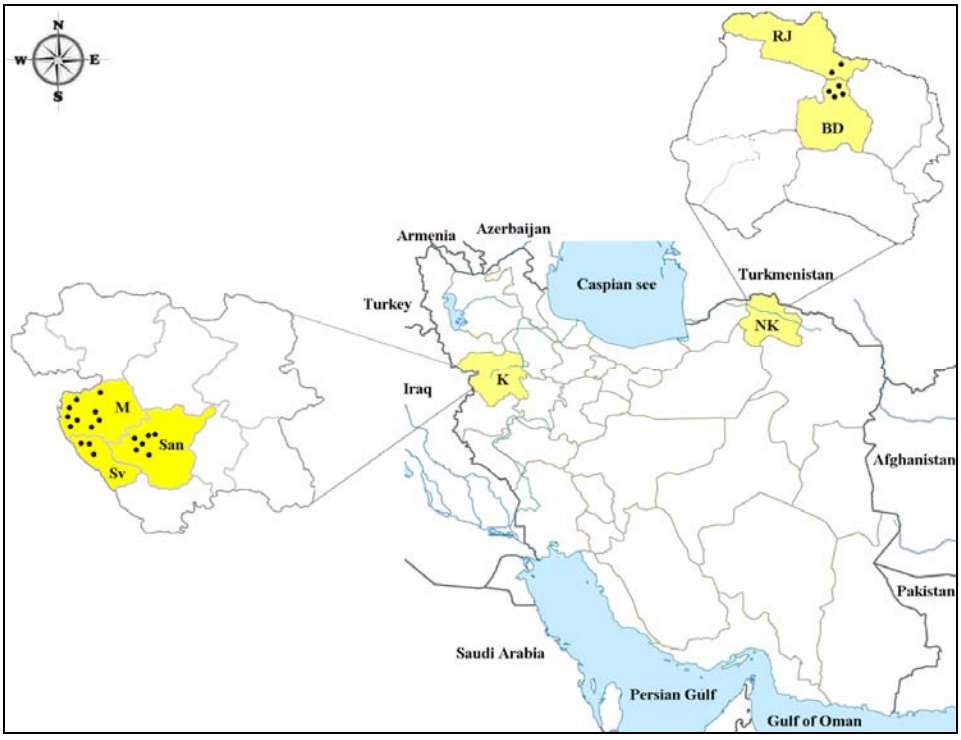


**Fig 1**: Location of rodent and flea collection sites in Kurdistan (K) province in west and North Khorasan (NK) Province in northeast of Iran. The dots denote the site of the villages sampled. M, Marivan; San, Sanandaj; SV, Sarvabad; BD, Bojnord; RJ, Razojergelan.

**Table 1:** The primers and probes used to detect Yersinia pestis in rodent spleen and fleas.

| Target gene | Primer and probe names | Sequence 5'-3' | PCR product length bp |
|---|---|---|---|
| *yihN*(Chromosome) | Chrom F<br>Chrom R<br>Chrom pro | CGCTTTACCTTCACCAAACTGAAC<br>GGTTGCTGGGAACCAAAGAAGA<br>Texas Red-TAAGTACATCAATCACACCGCGACCCGCTT-BHQ2 | 128 |
| *caf1*(pMT1) | pMT1 F<br>pMT1 R<br>pMT1 Pro | CCGTTATCGCCATTGCATTATTTGG<br>GCCAAGAGTAAGCGTACCAACAAG<br>FAM-AAGCACCACTGCAACGGCAACTCTT-BHQ1 | 194 |
| *Pla*(pPCP1) | pPCP1 F<br>pPCP1 R<br>pPCP1 Pro | ATTGGACTTGCAGGCCAGTATC<br>ATAACGTGAGCCGGATGTCTTC<br>FAM-AAATTCAGCGACTGGGTTCGGGCACA-BHQ1 | 144 |

## 3. Results

Totally 18 villages in Kurdistan and 6 villages in North Khorasan were investigated (Fig. 1 and Table 4). The locations in Kurdistan were former hotspots of plague and the villages in North Khorasan had no previous documented reports of infection but were parts of Khorasan that was an ancient plague focus.

Totally 84 rodents belonging to eleven species were trapped from Kurdistan province. Rodents of *Apodemus witherbyi* comprised 40.5 % of the trapped rodents and were the most abundant species in Kurdistan (Table 2). Members of genus *Meriones* as the main plague reservoirs constituted 12% of the trapped animals in Kurdistan. Most trapped animals were from Sanandaj (5 rodents/village), followed by Sarvabad (4.33 rodent/village), and Marivan (2.22 rodent/village). In North Khorasan, *Meriones persicus* exclusively was the only rodent species trapped (4.17 rodents/village) in the study area.

In Kurdistan, totally 95 fleas belong to four families, six subfamilies, and at least eleven species were collected from the trapped rodents (Table 2 and 3). Fleas of *Xenopsylla buxtoni* with 50.5% was the most prevalent flea in the region. In North Khorasan, 25 fleas belong to three species of *X. buxtoni, X. conformis nuttalli,* and *Paraceras spp* were collected from five infested rodents (Table 3). Members of *Xenopsylla* comprised 80% and 55.2% of the fleas collected from the north Khorasan and total study areas respectively. All Real-time PCR assays against the three genes of *yihN, caf1,* and *Pla* were negative for the existence of plague agent (*Y. pestis*) in the spleen and the fleas of the trapped rodents.

**Table 2**: Details of the trapped rodents and their fleas in the study area.

| Province | Species | No. of trapped Rodents (%) | No of fleas (No of infested rodent) | Flea/infested Rodent proportion |
|---|---|---|---|---|
| Kurdistan | *Apodemus witherbyi* | 34 (40.47) | 41 (5) | 8.2 |
| | *Apodemus mystacinus* | 3(3.57) | 16 (1) | 16 |
| | *Meriones persicus* | 5(5.95) | 12 (4) | 3 |
| | *Meriones libycus* | 2(2.38) | 0 (0) | 0 |
| | *Meriones teristrami* | 1(1.19) | 2 (1) | 2 |
| | *Meriones winogradovi* | 2(2.38) | 1 (1) | 1 |
| | *Microtus qazvinensis* | 13(15.47) | 4 (1) | 4 |
| | *Microtus socialis* | 19(22.61) | 18 (8) | 2.25 |
| | *Cricetulus migratorius* | 1(1.19) | 0 (0) | 0 |
| | *Mus macedonicus* | 3(3.57) | 1 (1) | 1 |
| | *Sciurus anomalus* | 1(1.19) | 0 (0) | 0 |
| | Subtotal | 84(100) | 95 (22) | 4.31 |
| North Khorasan | *Meriones persicus* | 25 (100) | 10 (5) | 2 |
| Total | | 109 (100) | 105 (27) | 3.88 |

**Table 3:** Details of the flea specimens collected from trapped rodents in the study area.

| Province | Family | Subfamily | Species | No. |
|---|---|---|---|---|
| Kurdistan | Pulicidae | Xenopsyllinae | *Xenopsylla buxtoni* | 48 |
| | | | *Xenopsylla spp* | 2 |
| | Ceratophyllidae | Ceratophyllinae | *Paraceras melio melis* | 23 |
| | | | *Paraceras spp* | 1 |
| | | | *Nosopsyllus iranus iranus* | 2 |
| | Leptopsyllidae | Mesopsyllinae | *Paradoxpsyllus microphtalmus* | 2 |
| | | | *Ctenophyllus rufescens* | 1 |
| | | Amphipsyllinae | *Amphipsylla rossica rossica* | 9 |
| | | Leptopsyllinae | *Leptopsylla segnis* | 1 |
| | Hystrichopsyllidae | Ctenophthalminae | *Ctenophtalmus spp* | 2 |
| | | | *Ctenophtalmus iranus persicus* | 4 |
| Subtotal | | 6 | 11 | 95 |
| North Khorasan | Pulicidae | Xenopsyllinae | *Xenopsylla buxtoni* | 5 |
| | | | *Xenopsylla conformis nuttalli* | 3 |
| | Ceratophyllidae | Ceratophyllinae | *Paraceras spp* | 2 |
| Subtotal | | 2 | 3 | 10 |
| Total | | | | 105 |

**Table 4:** Distribution of fleas collected on rodent species in the study areas.

| Province | District | Village | Rodent species | Flea species | Flea No |
|---|---|---|---|---|---|
| Kurdistan | Sanandaj | Tizh Tizh | *Meriones persicus* | *Xenopsylla buxtoni* | 1 |
| | | Shuyesheh | *Meriones persicus* | *Paradoxpsyllus microphetalmus* | 1 |
| | | | | *Xenopsylla buxtoni* | 8 |
| | | | | *Xenopsylla spp* | 1 |
| | | Khanghah | *Microtus qazvinensis* | *Xenopsylla buxtoni* | 1 |
| | | | | *Paraceras melio melis* | 2 |
| | | | | *Ctenophtalmus spp* | 1 |
| | | Khroseh | *Meriones winogradovi (Meriones teristrami)* | *Xenopsylla spp* | 1 |
| | | | *Meriones Persicus* | *Xenopsylla buxtoni* | 1 |
| | | | *Meriones teristrami* | *Nosopsyllus iranus iranus* | 2 |
| | Marivan | Nezhmar | *Microtus socialis* | *Ctenophtalmus iranus persicus* | 2 |
| | | | | *Amphipsylla rossica rossica* | 8 |
| | | | | *Xenopsylla buxtoni* | 1 |
| | | Almaneh | *Microtus socialis* | *Ctenophthalmus iranus persicus* | 1 |
| | | Garan | *Apodemus mystacinus* | *Paraseras melio melis* | 15 |
| | | | | *Paradoxpsyllus microphtalmus* | 1 |
| | | Kheyrabad | *Microtus socialis* | *Ctenophtalmus spp* | 1 |
| | | | | *Paraceras spp* | 1 |
| | | Sianav | *Apodemus witherbyi* | *Xenopsylla buxtoni* | 13 |
| | | | | *Paraceras melio melis* | 3 |
| | Sarvabad | Dezli | *Microtus socialis* | *Ctenophyllus rufescens* | 1 |
| | | Bahramabad | *Mus macedonicus* | *Ctenophthalmus iranus persicus* | 1 |
| | | | *Microtus socialis* | *Leptopsylla segnis* | 1 |
| | | | | *Amphipsylla rossica rossica* | 1 |
| | | Hawraman | *Microtus socialis* | *Xenopsylla buxtoni* | 1 |
| | | | *Apodemus witherbyi* | *Xenopsylla buxtoni* | 22 |
| | | | | *Paracera melio melis* | 3 |
| North Khorasan | Razojargalan | Baghliq | *Meriones persicus* | *Paracera spp* | 2 |
| | | | | *Xenopsylla conformis nuttalli* | 3 |
| | | Goinek | *Meriones persicus* | *Xenopsylla buxtoni* | 2 |
| | Bojnourd | Jami | *Meriones persicus* | *Xenopsylla buxtoni* | 2 |
| | | Mahnan | *Meriones persicus* | *Xenopsylla buxtoni* | 1 |
| Total | | | 8 | 12 | 105 |

## 4. Discussion

The present study was performed to evaluate potential activity or reemergence of the plague disease in two active and none active plague foci of Iran by testing the presence of *Y. pestis* and the diversity of its reservoirs and vectors. The present study showed that all the rodents and their corresponding fleas were clean of plague agent, however, diversity of both rodent reservoirs and flea vectors revealed potential situation for recurrence of plague in both locations.

Literature has shown that more than 200 wild rodent species have been infected by the plague agent in the natural foci around the world [4]. In Kurdistan, eleven rodent species were collected of which at least five species of *Meriones persicus, M. lybicus, M. tristrami, M. winogradov* and *Apodemus witherbyi,* are primary or secondary reservoirs or carrier of *Y. pestis* and play various epidemiological roles in the plague natural foci [1,9,13,14,26,27]. Other six rodent species that were found in Kurdistan were less frequent and may play a negligible role in the epizootic of the disease in the region [9]. However, some members of genus *Mus* [28], *Microtus* [29, 30], *Cricetulus* [31], and *Sciurus* [32,33] were found infected by plague agent in other parts of the world. These rodents may act as secondary and or incidental passersby and may represent auxiliary rodent sources of plague [26]. The eleven rodent species found in Kurdistan can be categorized into resistant, moderately resistant/susceptible, and susceptible animals. The resistant species act as enzootic (maintenance) hosts and susceptible ones act as epizootic (amplification) hosts [34].

In North Khorasan, we found entirely *M. persicus*, which is one of the main reservoirs of plague agent in Iran [14,19,35] Except for *A. witherbyi*, the proportion of fleas on the body of this rodent was much higher than other rodents, which is in agreement with earlier studies [14,35]. Since *Apodemus* spp and *Meriones* spp are known reservoirs for plague, and they carry vectors of plague, hence an actual potential reemergence of plague exist in both north Khorasan and Kurdistan.

Khorasan territory in north east of Iran is a natural breeding site for great gerbils, *Rhombomys opimus* [36–38]. This rodent is frequently infested by fleas of *Xenopsylla* [39], the main vector of the disease in the world. This rodent is known the main reservoir of plague agent in central Asia including Kazakhstan, Uzbekistan, Turkmenistan, Mongolia, and China [40–44]. Turkmenistan is neighboring to Khorasan territory in north east of Iran, and great gerbils occupied shared niches and climate condition in border line of Iran and Turkmenistan. However, yet there is no data indicating great gerbil infection in northeast of Iran and further researches are necessary to elucidate the role of this rodent in the plague cycles in northeast of the country.

Literature has shown that more than 80 flea species are known as proven vectors of plague [4]. To understand the epidemiology of plague in a given area, it is crucial to determine the flea species involved in plague transmission. The present study found twelve flea species in the study areas. More than 61% of the fleas were of genus *Xenopsylla* including *X. buxtani* and *X. conformis nuttalli,* which are the most important plague vectors in Iran and other parts of the world [14,19,35,40]. This high vector infestation of rodents in Kurdistan and North Khorasan, greatly increases the risk of

plague reemergence and transmission to humans. Most of the other flea species found in this study also have shown to be involved in plague endemic foci [45], for example, *Nosopsyllus* in India [46] and China [47], and *Amphipsylla* in Mongolia [47]. Also, *Leptopsylla segnis*, the mouse flea, is known as a weak vector of plague [48]. These fleas should be closely studied with new molecular methods to determine their contribution to the plague persistence in the regions.

So far, there is no information on the susceptibility/resistance status of flea vectors (*X. buxtani* and *X. conformis nuttalli*) of plague in Iran. We highly recommend further and continuous studies on insecticide and rodenticide susceptibility of vectors and rodents in the areas particularly in Kurdistan the active plague focus in Iran. The information on the flea vectors and rodent reservoirs are elementary to any plague control program.

## 5. Conclusion

This study revealed presence of various rodent species of *Meriones* and *Apodemus* genera as well as various flea species of *Xenopsylla* genus in the study areas. These rodents and fleas in turn are the known reservoirs and vectors for plague in Iran. Therefore, there is a real potential for the reemerging of plague disease in the studied areas.

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## 7. Conflict of Interest Statement

All authors have no conflict of interest.

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