

## Upsurge of Rodents' Population in a Rural Area of Northeastern Iran Raised Concerns about Rodent-borne Diseases

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**Introduction:** Rodents are the primary source of several zoonotic infectious diseases. The upsurge of rodents' population in Najaf Abad Village of Nishapur County, northeastern Iran in February 2014 raised the concerns about the outbreak of diseases such as plague and tularemia. This report discusses the lessons learned from the outburst of rodents' population in that village and represent as well the investigations performed by a dispatched team from Pasteur Institute of Iran for detection of potentially pathogenic agents in the entrapped rodents. **Methods:** In this study, different areas of Najaf Abad village were explored. The animals were identified based on morphological features using identification keys. Sera from all rodents were tested for plague and tularemia using ELISA and agglutination tests, respectively. **Results:** Seven captured rodents belonged to the family *Muridae Illiger*, 1811, and the species, *Mus musculus* Linnaeus, 1758 (n=4), *Meriones libycus Illiger*, 1811 (n=2), and *Nesokia indica* Gray, 1830 (n=1) were identified. All the rodents were negative for plague and tularemia. **Conclusion:** The outburst rodents' populations in this village that began in 2007 can be attributed to the old structure of the village, drought, and the disappearance of natural predators of rodents. Also, due to drought in the recent years, extensive agriculture was not possible, which might have led to the invasion of rodents to the human settlements. It is recommended that in such events, regular programs to be carried out under the supervision of an organization to achieve the best results in the shortest possible time. *J Med Microbiol Infect Dis*, 2017, 5 (1-2): 21-25.

**Keywords:** Rodent-borne Diseases, Plague, Tularemia, Zoonoses, Outbreaks.

### INTRODUCTION

Rodents comprise approximately 42 percent of all mammals' species and are present in all continents except Antarctica with the highest frequency and diversity among mammals [1]. Some species live in close contact with human populations in many parts of the world. These animals compete with the human for food and damage the grains and seeds before and after harvesting [1]. These animals also play a significant role in the transmission of many infectious diseases such as leishmaniasis, tularemia, plague, Hantavirus, leptospirosis, and listeriosis. The ectoparasites of rodents, e.g., fleas, ticks, lice, and mites, play a fundamental role in the transmission of some of the diseases mentioned above from rodents to humans [2]. The outbreak of rodent-borne diseases usually occurs following a sudden increase in the rodents' populations [3].

Plague caused by the bacterium *Yersinia pestis* is one of the most significant rodent-borne diseases worldwide. From the sixth to the nineteenth century, three plague pandemics led to the death of tens of millions of humans in Asia, Europe, Africa and South America. In some parts of the world, rodents are still the reservoirs of human plague and are considered a threat to the public health [4]. After decades of disappearance, plague outbreaks occurred in

Algeria (2003) and Libya (2009), and southern Afghanistan [5-7]. During 2014 to 2015, 308 cases of plague with 81 deaths (27% mortality) were reported from Madagascar [8]. Iran was stricken by a pandemic plague in 634-642 AD, and the disease was reported from the most parts of the country [9]. Moreover, several epidemics were reported in the Safavid era (1495-1735 AD) in the north, northwest, and northeast of Iran [10]. In 1877, an outbreak of plague was reported in a village of Sabzevar county in northeastern Iran. In 1878, plague struck another close village, 21 km of the location above and 42 km of the north of Sabzevar [11].

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In 1906, the first outbreak of plague was reported in Sistan and Baluchistan province, and in the last years, various outbreaks of plague were reported from southern parts of Iran; all of them were originated from India [12]. Recently, the persistence of the disease in Kurdistan province, western Iran was demonstrated by serology in wildlife [13].

The causative agent of tularemia, another significant zoonotic disease, is a gram-negative bacterium called *Francisella tularensis*. This agent is transmitted by arthropods such as ticks, fleas, and mosquitoes or through inhalation or ingestion of contaminated meat and water [14]. Tularemia causative agent has been isolated from 200 species of mammals, especially from rodents and rabbits [15]. The disease was reported from the north, northwest and northeast neighboring countries of Iran [16]. In a study in 1973, tularemia was reported in a hedgehog in southeastern Iran [17, 18]. Recently, tularemia infection was detected in high-risk human populations and rodents of Sistan and Baluchistan province, Southeast of Iran [19, 20].

After the rodents' invasion of Najaf Abad village, Firoozeh township of Nishapur County, in February 2014 center for communicable diseases control requested a survey of the incident. Regarding the previous plague and tularemia outbreak reports from eastern Iran and the adjacent areas, a research team from Pasteur Institute of Iran was dispatched to the area to investigate the potential rodents' infection to plague and tularemia. The present study focuses on the experiences and findings of this mission.

## MATERIAL AND METHODS

**Case report.** In February 2014, an unusual outburst of rodents' populations was reported in Najaf Abad village, Firoozeh County in Khorasan Razavi province. This village is located 12 km from the Firoozeh County (36° 3' to 36° 33' north and 58° 9' to 58° 41' east latitudes) with 602 residents, *i.e.*, 170 households who are mostly farmers and animal ranchers (Fig.1).

Concerns raised among the villagers after an increase in rodent populations. The traditional physical structure of the village was damaged, and some houses were evacuated (Fig. 2). The event caused political repercussions through the media and became a significant issue in Khorasan Razavi province and for the Ministry of Health and Medical Education [21-24]. The fight against the rodent population was performed several times by different organizations and villagers with different types of rodenticides and an electromagnetic device as well. On March 11, 2014, a team from Pasteur Institute of Iran was dispatched to the area to investigate the potential infection of rodents with plague and tularemia. Earlier, information about the incident was obtained from Nishapur Faculty of Medical Sciences, websites and news agencies. Supplementary information was gathered during a meeting with the director of health system network and political officials of Nishapur, Firoozeh County, and Najaf Abad village, as well as through interviews with the villagers.

**Sample collection.** A field visit was conducted to identify the appropriate locations in the village for installation of traps. Sampling was performed using Sherman traps (15×10×20 cm) with cucumbers, sausage, and Puff as preferable baits. Traps were installed in the evening and checked the next morning. Morphological characteristics of the hunted rodents including pelage coloration of the dorsal and ventral sides of the body and the tail, the length of head and body (HBL), tail length (TL), hind foot length (HFL) and ear length (EL) were recorded [25].

The ectoparasites of animals were brushed off into a sink filled with water, transferred to 70% ethanol, and transported to the laboratory for further investigation.

The rodents were anesthetized using a chloroform-impregnated cotton fabric, 2 ml of blood of their blood was taken with a syringe, and the serum was separated. Then the carcass was autopsied, the appearance of liver, kidney, and spleen was checked, and samples were taken for further investigations. The samples were transferred to the research center for emerging and reemerging infectious diseases of Pasteur Institute of Iran (National reference laboratory for plague, tularemia and Q fever).

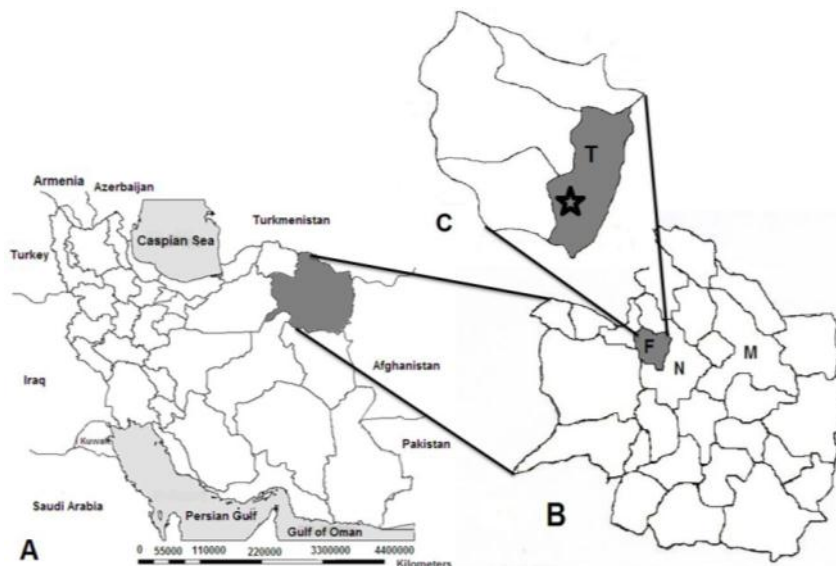
**ELISA test for detection of IgG antibodies against *Y. pestis*.** ELISA test was performed for detection of antibodies against the capsular antigen of *Y. pestis*. All antigens, positive and negative control sera, antisera and solutions required to perform ELISA test were provided by Pasteur Institute of Madagascar (WHO collaborating center for Plague). All steps of the test were performed according to the instructions given by Pasteur Institute of Madagascar. According to the kit instructions, ODs  $\geq 0.15$  for rodents were considered positive [26].

**Agglutination test for diagnosis of *F. tularensis*.** This test was done according to the manufacturer's instructions (Bioveta Company, Czech Republic) using an inactive bacterium with a positive control serum provided by the manufacturer. Agglutination tube test was prepared with 0.5 ml serial dilutions (1:10 to 1:160) of sera. Simply, 0.5 ml of diluted antigen (1:4) was added to each tube. The test was considered positive if a visible agglutination with clear supernatant fluid was seen after 20 h of incubation at 37°C followed by 1 h at room temperature. Agglutination at dilutions  $\geq 1:80$  was considered as positive while at the 1:40 dilution the result was considered suspicious.

## RESULTS

The collected data showed the outburst of rodents' populations in Najaf Abad village had begun in 2007.

Due to previous control measures by pesticides and the destruction of rodents' burrows, trapping was not very successful. Installing 306 traps in 35 points during three days resulted in entrapment of only seven rodents (four *Mus musculus*, two *Meriones libycus*, and one *Nesokia indica*). Serology tests for plague and tularemia were negative for all the animals. A flea belonging to the genus *Xenopsylla* was identified from one of trapped *M. libycus*.



**Fig. 1.** A, Map of Iran, the location of Khorasan Razavi province in northeastern Iran is shown in gray; B, the counties Mashhad (M), Nishapur (N) and Firoozeh (in gray, F); C, villages of Firoozeh county, Tahte Jolgeh (T) and Najaf Abad (\*)



**Fig. 2.** Effects of rodent infestation to houses in Najaf Abad village; ruined floor of residential houses

## DISCUSSION

The outburst of rodent's populations in the Najaf Abad village had led to the destruction of some parts of houses. The concern for villagers and officials was potential infections of rodents with specific diseases that might lead to outbreaks. Given the history of a plague outbreak in the area [11], the concern for the outbreak of this disease became serious for the health authorities. The governor, Ministry of Health and Medical Education, Ministry of Agriculture Iran, Department of Environment (DOE), private sectors and villagers had contributed in rodent control programs several times before the arrival of the Pasteur Institute of Iran investigation team, which led to entrapment of only a few rodents during the survey.

The serology tests for plague and tularemia were found to be negative for all the entrapped rodents. The collected rodents were identified as *M. libycus*, *N. indica*, and *M. musculus*. The last one is a commensal species distributed worldwide with the ability to grow fast in agricultural lands

with favorable conditions and becoming a significant pest [27]. During the upsurge of *M. musculus* in California, the density of this species was estimated to be 32400 animals per hectare. Similar events have also occurred in the former Soviet Union and Australia [27]. The species *M. libycus* is more active during the daytime. This rodent often lives near the bushes and along the edge of canals in plantations. This Jird is resistant to plague and together with the large Jird, *Rhombomys opimus*, are known to be important reservoirs of rural cutaneous leishmaniasis in most parts of the country [27]. *Nesokia indica*, a medium-sized rodent, tends to live beside streams and water channels and cause economic damage to agricultural products [27]. This species can also live in steppe areas and the regions with soft soil and vegetation. This rodent is reported to exist in some areas of the Middle East and most parts of Iran. This species is a reservoir of rural cutaneous leishmaniasis in southern Iran [27]. Reports from various countries show that the outburst of rodents' populations can lead to outbreaks or increase of some zoonotic diseases. For

example, an increase in rodents' populations in New Caledonia following a heavy rainfall led to an outbreak of leptospirosis [28]. Climate change was implicated as the cause of increased rodents' populations in China during 2005 to 2012, which was followed by an outbreak of hemorrhagic fever with renal syndrome. There is a significant correlation between climate changes (seasonal rainfall and temperature) and rodent's densities the outbreaks of diseases as well [29]. Our data from this study shows that the increase in rodent populations in this village had begun in 2007. This upsurge might be attributed to the old structure of the houses which provided shelter for rodents, drought, and the decreased populations of rodents' predators, e.g., raptors, owls, foxes, jackals, and snakes. Based on the statistical yearbook of Khorasan Razavi province, from the year prior to the upsurge, the pattern of cultivation had changed tremendously, e.g., the grains had decreased from 66.77 to 19%, beans from 1.44% to 1.2%, cucurbits from 67% to 21%, and forages increased from 7.96% to 24% and vegetables increased from 3.39% to 18% [30, 31]. This shift in cultivations might have contributed to the upsurge of rodents' populations.

Comparison of the temperature, humidity and rainfall of the region in the years before the upsurge with and the years of in which rodents' populations surged up showed no significant change; the average temperature reduced from 14.60°C in 2006 to 14.35°C in 2014. The humidity raised from the 49.3% in 2006 to 52.33% in 2014, and the precipitation reduced from 15.32 mm in 2006 to 13.64 mm in 2014 [30, 31].

In similar situations, activities such as transporting stockyards to the outside of the village, daily collection and disposal of the garbage, controlling the insects and rodents by expert persons, paying more attention to zoonotic diseases, training rural population about modes of transmission of zoonotic diseases and improvement of housing by cementing are recommended.

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## CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest associated with this manuscript.

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