

Original Research Article

Has Vector-Rodent relationship changed in Plague?: Experiences of plague affected hilly terrains of Himachal Pradesh, India.

Abstract

Background: The present study was conducted with objectives to elucidate the existence of sylvatic cycle of plague and to document the changing pattern of relationship between rodents and fleas.

Methods: Collection of samples was done from 15 sites (25 locations) from plague affected areas of Shimla district of Himachal Pradesh, India during 5 visits between June 2011 to July 2012, which were thereafter entomologically investigated in a BSL-3 laboratory.

Results: 57 fleas (3 species) from 243 rodents (3 species) were collected in domestic (n=16), peridomestic (n=3) and wild (n=6) locations. *Rattus rattus* (n=197) and *Nosopsyllus fasciatus* (n=43) was most frequently trapped rodent and flea species respectively. *Rattus rattus* (domestic rodent species) was also trapped from wild areas and *Nosopsyllus fasciatus* (domestic flea species) was also isolated from *Rattus norvegicus* (wild rodent species). One rare rodent species *Cornilurus albipes* was also found. Most common rodent-flea association was *Rattus norvegicus-Nosopsyllus fasciatus*. The recovery of rodents and flea was higher in months of June and December respectively.

Conclusions: The study concluded that sylvatic cycle persisted in study area and rodent and flea mixing was widely prevalent between domestic and wild area. This demands regular and intensive surveillance in plague prone hilly areas of Himachal Pradesh.

Key Words: Sylvatic cycle, plague, vector borne disease, surveillance

Introduction

Plague still remains a global threat due to its persistence in many countries around the world^[1]. It is highly lethal zoonotic emerging infectious disease which primarily circulates amongst wild rodent populations and is transmitted by fleas. The persistent circulation of coccobacillus, *Yersenia Pestis*, in wild reservoirs

(sylvatic plague) is responsible for occasional epidemics and pandemics of plague in human populations. On transmission to commensal rats (urban plague), commensal rats die in large numbers as they have less resistance to the disease. The fleas living on them then seek alternative hosts. *Y. pestis* taken up by the flea from an infected host in a blood meal undergoes a rapid multiplication in its stomach. The bacteria then form a viscous plug that blocks the stomach and prevents the flea from feeding normally. When the flea attempts to feed again, the blood comes up against the plug of bacteria and cannot enter the stomach. It is instead regurgitated by the flea back into the host. This process takes some bacteria with it, infecting the new host^[2]. Most of the cases of plague reported from countries in Asia, Africa and the Americas, are believed to have a close link to local wildlife reservoirs^[3-4].

Recurrent outbreaks of plague in India in recent past have exposed the vulnerability of the Indian population to epidemics. In Shimla district of Himachal Pradesh (H.P), a state of northern India, a small outbreak of primary pneumonic plague took place during February 2002. Sixteen cases of plague were reported with a case-fatality rate of 25% (4/16)^[5]. A previous outbreak in this region during 1983 was also suggestive of pneumonic plague (22 cases, 17 deaths). There had been several un-official reports of plague in the same region over past five decades^[6]. The factors and mechanism for re-emergence of plague is largely unknown, however there are many theories^[7], including survival of bacteria in soil^[8] or fleas^[9], and phases of lesser virulence of bacteria in hosts^[10]. A better understanding of the dynamics of sylvatic plague would improve the predictions of new outbreaks, thus making early warning and treatment possible. In the present study, we hypothesize the existence of sylvatic cycle of plague in a hilly region of India which had experienced epidemics of plague in past few decades.

Material and Methods

Study Design

It was a cross-sectional descriptive study

Study Settings

Shimla District is capital of mountainous state of Himachal Pradesh and is located in north-western ranges of Himalayas in Northern part of India. The population of Himachal Pradesh is 6.8 million (population of Shimla district is 0.8 million) and is spread over 55, 670 km². With an extreme variation in elevation ranging from 300 to 6000 meters above sea level, the temperature of Shimla District (31.61°N 77.10°E) range from -4 °C (25 °F) to 31 °C (88 °F) over the course of a year^[11]. The district is primarily rural and agriculture is the major economic activity, on which >90% of the population depends. The district has 25% land under forest reserve and rainfall (745 mm) mainly occurs in months of June till September. Soils are mainly low-pH (5.1-7.3) loams, rich in organic carbon, potassium, iron and manganese^[12].

Data collection

A total of 5 visits were made between June 2011 to July 2012 by the research team (medical-entomologist, veterinary doctor, microbiologist, animal attendant, field workers), each of one week duration, in 15 sites (25 locations: domestic (n=16), peridomestic (n=3) and wild (n=6)) of Shimla district of Himachal Pradesh (H.P), where either plague out breaks had occurred in recent past or were topographically conducive for rat flea's infestation. The places included visit-1 (Rohru, Hatkoti, Patsari), visit-2 (Mendali, Samala, Dodra-Pujali), visit-3 (Seeladesh, Kotkhai, Mural forest), visit-4 (Gari-Ganga Jungle, Parshal, Salayana), visit-5 (Nihari, Sarain forest, Deemo Davas). In the study, 'Domestic' locations were defined as locations within the human habitations or home; whereas, 'Peridomestic' locations were locations around human habitations like in fields and 'Wild' locations were locations within forests.

In each visit, a total of 48 Sherman traps (specialized rodent traps for live rodent trapping) on 3 grids, one grid at each of 3 sites, were placed per day for 6 days' trapping using sampling transect technique^[13]. Each grid or sampling transects consisted of 16 trap station (4 X 4) with each station separated by 20 meters. The traps were laid with bait (Bread, onion, tomato, chappati and butter) in domestic, peridomestic and wild areas (areas of thick and dense vegetation). The traps were landed during dusk time and retrieved next day before the first light. The same grids were placed for six consecutive nights.

Trapped animals were placed in anaesthetized plastic bags and brought back to a laboratory for further processing. Each plastic bag was inverted over an enamel tray to dislodge its contents. The ectoparasite collection followed host search method using the protocol by Soliman et al ^[14] and Walker ^[15]. The anaesthetized rodents were ear-tagged, sexed, identified upto species level and screened for ectoparasites by using fine wet combing method. Additional fleas were collected from the plastic bag used in handling the animal. Flea were preserved in 2% NS (normal saline) and examined under a dissecting microscope upto species level. The rodents and flea samples were brought to a BSL-3 central laboratory in a tertiary care hospital, PGIMER Chandigarh for further investigation and identification. They were stored in medical microbiology lab at optimum temperature. The standard operating procedures (SOP) were designed based on the standardized guidelines by Committee for Purpose of Control and Supervision of Experiments on Animals (CPCSEA) ^[16]. The rodents were later released at their capture sites after data collection.

Analysis and Statistics

The rodent positivity rate was defined as the number of rodents trapped in the traps divided by number of traps laid multiplied by 100. Similarly, the flea positivity rate was defined as the number of flea collected on rodents divided by number of rodents collected multiplied by 100 ^[17]. The data was double entered in Excel for windows. Descriptive analysis was performed. Chi-square test or Fishers exact test (wherever applicable) was used to find association between trap positivity, rodent positivity and flea positivity with the time (month) of year.

Administrative approvals and Ethical permission

Prior permission was obtained from Directorate General Health Services, State of Himachal Pradesh and Director, Wild Life Authority, Himachal Pradesh. The project was approved by Institutional Ethics Committee of PGIMER, Chandigarh.

Results

A total of 57 fleas (3 species) were collected from 243 rodents (3 species). *Rattus rattus* (n=197) was most frequently trapped rodent followed by *Rattus norvegicus* (n=45) and *Cornilurus albipes* (n=1). With regards to flea, *Nosopsyllus fascitus* (n=43) was most commonly encountered flea followed by *Epitedia wenmanni* (n=13) and *Leptopsylla segnis* (n=1).

Rodent and flea mixing among domestic and wild area was observed. *Rattus rattus* (domestic rodent species) was trapped from all areas including peridomestic and wild areas. Similarly, *Nosopsyllus fascitus* (domestic flea species) was isolated from *Rattus norvegicus*(wild rodent species) besides *Rattus rattus*. *Epitedia wenmanni* and *Leptopsyllas segnis* was exclusively located in wild areas on *Rattus norvegicus* and *Cornilurus albipes* (White Footed Rat) respectively. (Table-1, 2 and 3)

The overall rodent positivity rate was 32.5%. It was more for *Cornilurus albipes* (100%) and *Rattus rattus* (38.5%) as compared to *Rattus norvegicus* (19.1%). 57.8% (33/57) of total flea were recovered from *Rattus norvegicus* with 73.3% (33/45) flea load, whereas, *Rattus rattus* harbored 40.3% (23/57) flea with flea load of 11.7% (23/197). One *Leptopsylla segnis* was isolated from *Cornilurus albipes* with flea load of 100% (1/1). (Table-4)

The most common rodent-flea association was observed in *Rattus norvegicus-Nosopsyllus fascitus* with infestation rate of 44% (20/45) followed by *Rattus norvegicus-Epitedia wenmanni* with infestation rate of 29% (13/45) (Table - 5)

The flea infestation rates were more in month of December (73%) followed by month of August (11%). The recovery of rodents was significantly more in month of June and least in September as compared to other months. Based upon Fisher Exact test, a statistical significant difference ($p<0.01$) was observed for trap positivity, rodent positivity and flea positivity across different months of a year. (Table-6)

Discussion

The study confirms our hypotheses that the sylvatic cycle of plague exists in a hilly areas of Himachal Pradesh, India. Further, inter-mixing of rodent and flea species occurs between domestic and wild areas.

In addition, few rare species of fleas were found in the study area, which normally do not exist in this region. All these findings points towards the fact that there is circulation of plague bacilli in the region which have crossed the barrier and has moved nearer to human population from wild settings.

In India and other tropical nations, the plague season generally starts during the warmer rainy months of July till August. The situation is quite similar to central highlands, where most of the cases occur during the warmer rainy months of October and February^[18]. In present study too, maximum catches of rats and fleas were found in the warmer rainy months of June and August. The probable reason could be that the high moisture (humidity over 60%) favors breeding of fleas as well as infected fleas may be alive for months together in regions where moisture of environment is high. Moreover, the onset of heavy rain in month of August could drive rats into human dwellings, which may increase the likelihood of human exposure to infected rats or their fleas. Thus, the population of the rat fleas builds up quickly in these favorable conditions leading to plague out breaks^[19].

In our study, flea infestation rate was more in colder months of December, which could be linked to the fact that snowfall could also drive rats from forests into human dwellings for the food, thus increasing the likelihood of human exposure to infected rats or their parasites. All these circumstances lead to commingling (inter-mixing)^[20-21]. Epidemic ceases when the temperature exceeds 26.7°C and saturation deficit is more than 10.2 mb by causing high mortality among larvae and reducing the fecundity and survival of adult fleas^[22]. Bacot stated that with nearly saturated air at 45-50°F (approx-7-10°C) unfed *N. fasciatus*(Bosc) survived for 125 days; *X. cheopis* (Roth) survived for 38 days. The extremes of temperatures (more than 35°C and less than 10°C) inhibit the growth of the developmental stages of flea. The sensitivity of adult and developing fleas to low humidity and to high temperature extremes may be a principal reason why fleas occur in large numbers on animals that lives in burrows and nests^[23]. Mellanby stated that pupation of *X. cheopis* at fixed relative humidity of 60% occurs in 8 days at 18°C, 6 days at 22°C and 4 days at 29-35°C^[24].

Globally *X. cheopis* has been reported as the major vector of plague as it showed the highest rate of blockage (58%), highest transmission rate and lowest rate of eliminating *Y. pestis* from its body^[25]. However, in our study, *X. cheopis* flea was not recovered on rodent population. Instead, *N. fasciatus* was found to be a dominant vector. The results of present study are in consonance with the San Francisco plague epidemic in 1907, where in, *N. fasciatus* was the major flea species (68.1%) recovered from rats^[26]. *N. fasciatus*, is not host-specific and is usually present on peridomestic and domestic rodents. This characteristic leads to its long survival in the environment. Both the larva and adult of *N. fasciatus*, survive best in slightly damp piles of rubbish where it is cool and humid. In another outbreak in Calcutta, India in 1925, the flea population constituted *X. asita* (66%) and *X. cheopis* (34%). After the bubonic plague outbreak during 1994 in the states of Maharashtra, Gujarat, Uttar Pradesh and Union Territory of Delhi seven species of rodents, viz. *Rattus rattus*, *R. norvegicus*, *Mus. musculus*, *Tatera indica*, *Suncus murinus*, *Bandicoota bengalensis* and *B. indica* and two flea species viz., *X. cheopis* and *X. asita* were found^[27].

The main occupation of the people in the studied area was agriculture. Most of their activities are confined during months of June to September (monsoon period) primarily because of cropping pattern (peanuts, maize, potato and apple) in this season. This also tends to increase their chances contact with the infected flea in the fields, which are already in high proportions due to optimal weather conditions. This additionally enhances the risk of transmission of plague.

One of the factors contributing to increase commingling is urbanization. It is reported that the natives of study area used to go for hunting in jungles during winter season and stay in caves for days together. Simultaneously they also indulge in collection of 'gucchhi' a type of mushroom found in forest earn their livelihood. This way, possibly they usually come in contact with the wild rodents that were infected with fleas. The activity of staying in jungles for long time may also lead to influence the social behavior of vertebrates, where animals tend to move out of the homes (jungles) to domestic populations. This can break up the social structure and can cause long-term effects on the demography of hunted as well as

nearby populations, amplifying the impact of hunting. Poaching, deforestation, forest fire, construction of roads /dams, destruction of eco-system (food-chain and food-web) are other the human interventions which increase the chances of inter-mixing of rodents / rat fleas species, increasing the risk of human plague. Apart from this, many demographic and sociological factors also play a critical role in determining the dynamics of disease transmission. This includes changes in land use, agricultural methods, residential patterns, human behavior, and vector control activities.

The present article is a part of Indian Council of Medical Research Adhoc Research Grant. The authors have published other related aspects of plague under the project. The authors have established a potential link between source of plague infection in forests and native population of Himachal Pradesh and other states of India^[28]. They had also suggested role of lifestyle and other socio-epidemiological factors in the causation of outbreak of plague^[29-30]. The authors have also concluded that Rapid Diagnostic Kit (RDT) is a valid and reliable test for detection of plague outbreaks in field settings^[31].

Conclusions

With the existence of sylvatic cycle, including the number and variety of vector and flea species involved, and intermixing of host-parasite relationship, it is unlikely that plague will ever be eradicated by human endeavors. These recurrent plague epidemics in India remind us that its resurgence merely requires favorable socio- environmental and public health conditions for the vector and fleas. However, the present study does not explain about disappearance of *X cheopis* from the environment and existence of *N fasciatus* as a primary vector in place of *X. Cheopis.*, for which further studies are needed. The role of climatic or ecological factors needs to be further studied.

Ethical approval: The project was approved by Institute Ethics Committee, PGIMER, Chandigarh.

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Conflict of Interest: None

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Table – 1: Rodent and Flea species collection from various sampling locations in Shimla, Himachal Pradesh, India(June 2011 to July 2012)

Sampling locations (n=25)

Rodent species	Domestic (n=16)																Peridomestic (n=3)			Wild (n=6)					
	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X	O
<i>Rattusrattus</i> (n=197)	n=50	n=5	n=3	n=5	n=5	n=2	n=1	n=15	n=11	n=12	n=13	n=5	n=11	n=5	n=11	n=13	n=9	n=4	n=10						n=7
<i>Rattusnorvegicus</i> (n=45)											n=2									n=5	n=13	n=8	n=11	n=6	
<i>Cornilurusalbi</i> (n=1)																					n=1				
Flea species																									
<i>Nosopsyllus</i> <i>sciatus</i> (n=43)	n=6				n=2						n=8	n=4						n=2	n=2				n=16	n=3	
<i>Epitediawenmanni</i> (n=13)																					n=9		n=4		
<i>Leptopsylla</i> <i>segnis</i> (n=1)																					n=1				

A = Rohru, B = Hatkoti, C = Patsari, Mendali, D = Kaloti, E = Samala, F = Salayana, G = Nihari, H = Kotkhai, I = Deemo, J = Davas, K = Diadu, L = Maila, M = Dhamwari, N = Kharshali,

O = Ruhidar, P = Aunti, Q = Dodra, R = Dora-Pujali, S = Sheeladesh, T = Mural, U = Gari Ganga, V = Sarain, W = Tangnu, X = Gajiyani

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Table – 2: Location wise Rodent-Flea relationships in study area(June 2011-July 2012)

Rodent species	Number	Flea species	Number (%)	Place
<u>Rattusrattus</u>	197	<u>Nosopsyllusfascitus</u>	23 (11.6)	Domestic (Samala)
<u>Rattusnorvegicus</u>	45	<u>Nosopsyllusfascitus</u>	20 (44.4)	Wild (Sarain)
		<u>Epitediawenmanni</u>	13 (28.8)	Wild (Gari Ganga)
<u>Cornilurusalbipes</u>	1	<u>Leptopsyllassegnis</u>	1 (100)	Wild (Gari Ganga)

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Table – 3: Location wise flea infestation rate data for rodents (June 2011-July 2012)

				Flea species recovered		
Setting	Rodent species	No.	No. hosts infested (%)	<u>Nosopsyllusfascitus</u>	<u>Epitediawenmanni</u>	<u>Leptopsylla.segnis</u>
Domestic	<u>Rattusrattus</u>	170	40 (23.5%)	19 (0.11)		
	<u>Rattusnorvegicus</u>	5	2 (40%)	1 (0.2)		
Peri-domestic	<u>Rattusrattus</u>	23	14 (60.8%)	4 (0.17)		
Wild	<u>Rattusnorvegicus</u>	45	38 (84.4%)	19 (1)	13 (0.5)	
	<u>Cornilurusalbipes</u>	1	1 (100%)			1 (1)

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Table – 4: Rodent and Flea positivity rates in the sampling locations(June 2011-July 2012)

Species	No. rodents found	**Rodent +vity Rate	No. fleas collected	*Flea +vity Rate
<u>Rattusrattus</u>	197	38.5%	23	11.7%
<u>Rattusnorvegicus</u>	45	19.1%	33	73.3%
<u>Cornilurusalbipes</u>	1	100%	1	100%
Total	243	32.5%	57	23.4%

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****Rodent +vity Rate:** Total No. of Rodent found/ Traps Laid×100

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***Flea +vity Rate:** Total No. of Fleas Collected/ Total No. of Rodents Collected×100

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Table – 5: Flea infestation of rodents collected in various sampling locations (June 2011-July 2012)

Rodent species	Total number of fleas collected (no. fleas/hosts examined)		
	<u>Nosopsyllusfascitus</u>	<u>Epitediawenmanni</u>	<u>Leptopsylla.segnis</u>
<u>Rattusrattus</u> (197)	23 (23/197=0.12)	-	-
<u>Rattusnorvegicus</u> (45)	20 (20/45=0.44)	13 (13/45=0.29)	-
<u>Cornilurusalbipes</u> (1)	-	-	1 (1/1=1)

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Table – 6: Monthly trends in flea infestation rates, by host species and location in study area (June 2011-July 2012)

Average no. fleas per host examined (total hosts examined)								
		Rohru, Hatkoti, Patsari, Mendali, Kaloti, Samala, Kotkhai, Dodra, Dodra- Pujali, Sheeladesh	Gari Ganga Forest, Mural Forest, Gari Ganga	Salayana, Nihari	Davas, Demo, Sarain Forest	Tangnu, Diadu, Maila	Kharshali, Dhamwari, Gajiyani	Ruhidar, Aunti
Flea species	Host species	June, 2011	August, 2011	September, 2011	October, 2011	December, 2011	February, 2012	July, 2012
<i>Nosopsyllus fasciatus</i>	<i>Rattus rattus</i>	0.11 (106)	0 (5)	0 (3)	0 (23)	0.73 (15)	0 (16)	0 (30)
	<i>Rattus norvegicus</i>				0.5 (8)	1.31 (13)	0.43 (7)	
<i>Epitediawenmanni</i>	<i>Rattus norvegicus</i>		0.5 (18)			0.75 (4)		
<i>Leptopsylla segnis</i>	<i>Cornilurus albipes</i>		1 (1)					

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