

## Vital Surveillances

# Rodent Ecology and Etiological Investigation in China: Results from Vector Biology Surveillance — Shandong Province, China, 2012–2022

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## ABSTRACT

**Introduction:** Rodents are hosts of a wide range of zoonotic disease pathogens which threaten human health. However, comprehensive investigations of rodent ecology and etiology in Shandong are lacking. Thus, we aimed to analyze rodent ecology and infection with relevant pathogens in Shandong Province, China.

**Methods:** Rodent survey data collected from 2012 to 2022 in Shandong Province were used in this study. Rodents captured from 2020 to 2022 were identified to species and tested for pathogens.

**Results:** From 2012 to 2022, 4,145 rodents were captured, with an average capture rate of 0.70%. High capture rates were observed in rural residential areas and other habitats, such as farmland and forestland. *Rattus norvegicus* (*R. norvegicus*) was the dominant species, followed by *Mus musculus* (*M. musculus*). The regions with the highest capture rates of *R. norvegicus* were Dongying (0.82%) and Heze (0.63%), while *M. musculus* was more prevalent in Dongying (0.81%) and Weihai (0.56%). Rodent capture rates were highest between March and September. The positive detection rates of Hantavirus (HV), *Leptospira interrogans* (*L. interrogans*), *Rickettsia typhi* (*R. typhi*), *Anaplasma phagocytophilum* (*A. phagocytophilum*), and *Francisella tularensis* (*F. tularensis*) in rodents were 2.58%, 1.10%, 0.94%, 0.16%, and 0.19%, respectively.

**Conclusions:** The rodent capture rate in human habitation environments has trended downward in Shandong Province, with *R. norvegicus* and *M. musculus* being the dominant species. Rodent infection risk from HV, *L. interrogans*, and *R. typhi* showed seasonal variation. Strengthening rodent surveillance and maintaining a low capture rate of host animals could be pivotal for preventing and controlling relevant rodent-borne diseases in high-risk areas.

Rodents are among the most speciose mammals in the world, closely interacting with the human ecosystem (1). Rodents are well-known reservoirs and hosts for numerous infectious diseases (e.g., hemorrhagic fever with renal syndrome (HFRS), leptospirosis, scrub typhus, and plague) and play an important role in their transmission and spread (2). With the risks of global warming, changes in the ecological environment, increased trade, and population mobility, rodents and their infectious diseases present a serious threat to public health in China (3–4). Since the discovery of HFRS cases in Shandong Province in 1962, its incidence has long been among the highest in the country (5). We analyzed the trends of rodent species, densities, seasonal fluctuation, and pathogen infection in Shandong Province to shed light on providing early warning and relevant rodent and rodent-borne disease control measures.

## METHODS

Rodent surveillance data from 2012 to 2022 were obtained from surveillance sites in 16 regions of Shandong Province. Survey habitats were classified as urban residential areas, rural residential areas, and key industries (catering, food production and sale, construction sites, slaughterhouses, breweries, etc.). Year-round surveillance was conducted from 2012 to 2015. In 2016, the National Vector Surveillance Implementation Plan was issued, and surveillance was carried out in odd-numbered months from 2016 to 2022.

From 2020 to 2022, rodents captured for pathogenic surveillance were distributed across 12 regions in Shandong Province. The surveying time and habitat were consistent with the ecological surveying of rodents. The methods of nucleic acid extraction and pathogen detection were as previously described (6). Briefly, DNA was extracted from the liver, kidney, and

spleen using the QIAGEN RNeasy Mini Kit (QIAGEN, Germany) and analyzed for the detection of *Leptospira interrogans* (*L. interrogans*), *Rickettsia typhi* (*R. typhi*), *Orientia tsutsugamushi* (*Ot*), *Anaplasma phagocytophilum* (*A. phagocytophilum*), and *Francisella tularensis* (*F. tularensis*) via real-time PCR assay (qPCR). RNA was extracted using the QIAGEN RNeasy Mini Kit (QIAGEN, Germany). RNA extractions from the liver, kidney, and spleen were analyzed for the detection of Dabie bandavirus (DBV), and RNA extracted from the lung was analyzed for the detection of HV via real-time reverse-transcriptase PCR assay (qRT-PCR). The positive status of specimen tests is shown in Supplementary Table S1 (available at <https://weekly.chinacdc.cn/>). Count data were analyzed using the  $\chi^2$  test and Fisher exact tests with R software (version 4.0.2; TUNA Team, Tsinghua University, Beijing, China). A  $P < 0.05$  was considered statistically significant.

## RESULTS

From 2012 to 2022, a total of 593,563 effective traps (cages) were deployed at rodent surveying sites in Shandong Province, resulting in the capture of 4,145 rodents. The average rodent capture rate was 0.70% [95% confidence interval (CI): 0.68%–0.87%]. The highest average annual rodent capture rate was 1.00% in 2012, while the lowest was 0.51% in 2022. Overall, rodent densities displayed a fluctuating downward trend ( $Z = -3.581$ ,  $P < 0.001$ ) (Figure 1A). Among the rodent surveying sites, Dongying (1.99%) had the

highest rodent capture rate, followed by Heze (1.23%), while Binzhou (0.29%) had the lowest (Supplementary Table S2, available at <https://weekly.chinacdc.cn/>); this difference was statistically significant ( $\chi^2 = 981.33$ ,  $P < 0.001$ ).

From 2012 to 2022, the capture rate of rodents in rural residential areas and other habitats (farmland and woodland) was relatively high, with *Rattus norvegicus* (*R. norvegicus*) as the dominant species (Table 1). In recent years, the proportion of *R. norvegicus* and *Mus musculus* (*M. musculus*) remained relatively high, while the proportion of *A. agrarius* and other species increased (Figure 1B).

The rodent capture composition differed among the 16 districts in Shandong Province ( $\chi^2 = 1023$ ,  $P < 0.001$ ). Areas with high capture rates of *R. norvegicus* were mainly distributed in Dongying (0.82%) and Heze City (0.63%). Capture rates of *M. musculus* were relatively high in Dongying (0.81%), Weihai (0.56%), and Heze (0.56%). Capture rates of *A. agrarius* were relatively high in Dongying (0.30%) and Qingdao (0.11%). Most *R. flavipectus* were captured in Tai'an (0.05%) and Zibo (0.03%) cities (Supplementary Table S2).

From 2012 to 2022, a seasonal trend was observed in Shandong Province, with a higher rodent capture rate from March to September (Figure 2). However, some regional differences were noted. The regions with high rodent capture rates in winter and spring were mainly Jinan, Zaozhuang, Yantai, Weifang, Jining, Linyi, Dezhou, and Binzhou cities. The regions with high rodent capture rates in summer and autumn were

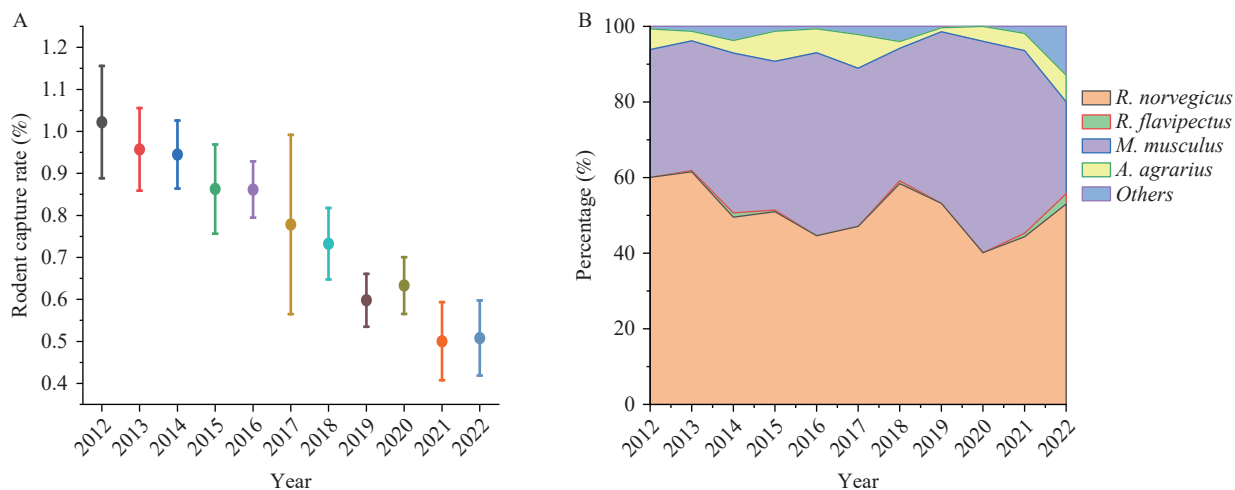


FIGURE 1. Annual trends in rodent capture rate and composition ratio from 2012 to 2022. (A) The trend of rodent capture rates; (B) Annual changes in rodent capture composition.

Note: The error bars represent a 95% confidence interval of the rodent capture rate.

TABLE 1. Rodent densities and rodent pathogen positive rate (%) in different habitats.

Item	Rural residential areas	Urban residential areas	Key industries	Other habitats*	Total
<b>Rodents</b>					
<i>Rattus norvegicus</i>	986	525	561	47	2,119
<i>Rattus flavipectus</i>	17	6	11	0	34
<i>Mus musculus</i>	835	448	340	2	1,625
<i>A. agrarius</i>	128	9	70	7	214
Others*	81	26	46	0	153
Effective traps	206,056	197,847	183,217	6,443	593,563
No. of rodents	2,047	1,014	1,028	56	4,145
Capture rates (%)	0.99	0.51	0.56	0.87	0.70
<b>Pathogens</b>					
<i>Hv</i>					
No. rodents	1,305	90	959	394	2,748
No. positive	29	0	4	38	71
Positive rate	2.22	0	0.42	9.64	2.58
<i>BDV</i>					
No. rodents	1,323	90	962	394	2,769
No. positive	0	0	0	0	0
Positive rate	0	0	0	0	0
<i>Leptospira interrogans</i>					
No. rodents	1,226	88	959	374	2,647
No. positive	15	0	12	2	29
Positive rate	1.22	0	1.25	0.53	1.1
<i>Rickettsia typhi</i>					
No. rodents	412	32	489	29	962
No. positive	8	0	1	0	9
Positive rate	1.94	0	0.2	0	0.94
<i>Ot</i>					
No. rodents	501	20	474	47	1,042
No. positive	0	0	0	0	0
Positive rate	0	0	0	0	0
<i>Anaplasma phagocytophilum</i>					
No. rodents	276	2	339	7	624
No. positive	0	0	1	0	1
Positive rate	0	0	0.29	0	0.16
<i>Francisella tularensis</i>					
No. rodents	241	2	271	6	520
No. positive	1	0	0	–	1
Positive rate	0.41	0	0	–	0.19

Abbreviation: Hv=hantavirus; BDV=dabie bandavirus; Ot=*orientia tsutsugamushi*.

\* Other habitats contain farmland, forestland, etc.

“–” No testing was conducted.



FIGURE 2. Heat map of seasonal changes in rodent surveillance in Shandong Province, China, from 2012 to 2022.

mainly Zibo, Tai'an, and Weihai cities. The seasonal fluctuation of rodent capture rates in Dongying and Heze cities was not significant.

A total of 2,769 rodents (including shrews) were captured for the detection of rodent-borne pathogens. Among them, 2,748, 2,769, 2,647, 962, 1,042, 624, and 520 rodents were screened for HV, DBV, *L. interrogans*, *R. typhi*, *Ot*, *A. phagocytophilum*, and *F. tularensis*, respectively. Of the seven pathogens screened, HV had a positive rate of 2.58%, the highest positive rate in rodents captured in Shandong Province. The annual changes in the detection results of the various pathogens are shown in Supplementary Table S3 (available at <https://weekly.chinacdc.cn/>).

HV was detected in *R. norvegicus* (4.58%), *A. agrarius* (2.29%), and *R. flavipectus* (0.85%). *L. interrogans* was detected in *M. musculus* (1.80%) and *R. norvegicus* (0.90%). *R. typhi* was detected in *M. musculus* (2.80%) and *R. norvegicus* (0.44%). *A. phagocytophilum* was detected only in *S. murinus* (20%), and *F. tularensis* was detected only in *R. norvegicus* (0.56%). DBV and *Ot* were not detected in any captured rodents (Table 2).

The rodent-borne pathogens found in rural

residential areas were HV (2.22%), *L. interrogans* (1.22%), *R. typhi* (1.94%), and *F. tularensis* (0.41%). Pathogens found in rodents in key industries were HV (0.42%), *L. interrogans* (1.25%), *R. typhi* (0.20%), and *A. phagocytophilum* (0.29%). Pathogens detected in rodents captured in farmland and forestland were HV (9.64%) and *L. interrogans* (0.53%). None of the above seven pathogens were detected in urban residential areas (Table 1).

The total HV-positive rate was 2.58%, with higher rates found in Weifang (15.53%) and Qingdao (3.50%). The total *L. interrogans*-positive rate was 1.10%, with higher rates found in Jinan (9.00%) and Dongying (2.10%). The *R. typhi*-positive rate was only found in Jinan (9.00%). *A. phagocytophilum* and *F. tularensis* were found in one rodent from Jinan (1.00% each) (Supplementary Table S2).

## CONCLUSIONS

In this study, the average rodent capture rate in Shandong Province was 0.70% from 2012 to 2022, relatively higher than in southern China but lower than in northern China (7–8). The overall rodent

TABLE 2. Positive rate of the pathogens in different species of rodents (%).

Rodent species	Hantavirus			Dabie bandavirus			L. interrogans			R. typhi			Orientia tsutsugamushi			A. phagocytophilum			F. tularensis		
	No. rodents	No. positive	Positive rate	No. rodents	No. positive	Positive rate	No. rodents	No. positive	Positive rate	No. rodents	No. positive	Positive rate	No. rodents	No. positive	Positive rate	No. rodents	No. positive	Positive rate	No. rodents	No. positive	Positive rate
<i>R. norvegicus</i>	1,420	65	4.58	1439	0	0.00	1,335	12	0.90	453	2	0.44	538	0	0.00	261	0	0.00	177	1	0.56
<i>M. musculus</i>	950	1	0.11	951	0	0.00	946	17	1.80	250	7	2.80	245	0	0.00	191	0	0.00	186	0	0.00
<i>A. agrarius</i>	131	3	2.29	131	0	0.00	120	0	0.00	53	0	0.00	64	0	0.00	21	0	0.00	19	0	0.00
<i>R. flavipectus</i>	235	2	0.85	236	0	0.00	236	0	0.00	200	0	0.00	187	0	0.00	145	0	0.00	132	0	0.00
<i>S. murinus</i>	9	0	0.00	9	0	0.00	9	0	0.00	5	0	0.00	5	0	0.00	5	1	20.00	5	0	0.00
<i>Niviventer bukit</i>	2	0	0.00	2	0	0.00	-	-	-	-	-	-	2	0	0.00	-	-	-	-	-	-
<i>Cricetulus</i>	1	0	0.00	1	0	0.00	1	0	0.00	1	0	0.00	1	0	0.00	1	0	0.00	1	0	0.00
Total	2748	71	2.58	2769	0	0.00	2647	29	1.10	962	9	0.94	1042	0	0.00	624	1	0.16	520	1	0.19

“-” : No test..

capture rate is decreasing, potentially due to sanitary city initiatives and enhanced rodent breeding site cleanup in rural residential areas (9). Rodent capture rates varied geographically; higher capture rates were primarily in eastern (Weihai, 1.22%; Qingdao, 0.93%), southwestern (Heze, 1.23%), and northern (Dongying, 1.99%) Shandong Province, largely consistent with the high HFRS prevalence in Shandong Province (5).

In our study, *R. norvegicus* was the major species, followed by *M. musculus*, *A. agrarius*, and *R. flavipectus*. These results were similar to those of an investigation in Zhejiang Province (7). This finding may be related to the habits and adaptations of various rodent species and suggests that *R. norvegicus* and *M. musculus* were the key rodent species for rodent control in human residential areas (10).

A previous study showed that the rodent capture rate increased during the spring breeding season, with two insignificant capture rate peaks in the spring and autumn in Shandong Province (11). In contrast, this study found that rodent seasonal fluctuation increased from winter to spring, peaked in May, July, and September, and did not exhibit two distinct capture rate peaks. A previous study indicated that monthly HFRS outbreaks significantly correlated with the rodent capture rate 2 months earlier (12), which was generally similar to HFRS incidence in Shandong Province (5).

Our results indicate that four of the seven pathogens tested were detected in rodents, with the exception of DBV and *Ot. HV* and *L. interrogans* were the most prevalent. *R. norvegicus* and *A. agrarius* were the main reservoirs of HV (13). The total HV-positive rate in rodents in our study was 2.58%, with HV circulating mainly in *R. norvegicus* and *A. agrarius*, with infection rates of 4.58% and 2.29%, respectively. The proportion of *M. musculus* carrying HV was very low in this study. Farmland and forestland (9.64%) were the key habitats for the detection of HV in rodents. Five regions had HV-positive results, with Weifang exhibiting a positive rate of over 15%. A previous study also found that farmers account for the largest proportion of all HFRS cases (13). One study showed that *L. interrogans* had the highest detection rate of 12.28% and tested positive in almost all common rodent species in Zhejiang Province (7). We found that *L. interrogans* circulated mainly in *M. musculus* and *R. norvegicus*, with infection rates of 1.80% and 0.90%, respectively. Rural residential areas and key industries were habitats with a high positive rate of *L. interrogans*.



The northern cities of Shandong (Jinan, Dongying, and Binzhou) were the key regions for the prevention and control of leptospirosis. These findings suggest that farmers and workers in farmland and forests are at high risk of rodent-borne diseases. It is necessary to strengthen surveillance-based integrated rodent control and enhance the personal protection of rural field workers in areas with a high risk of rodent-borne pathogen infection.

*R. typhi*, *A. phagocytophilum*, and *F. tularensis* were found positive only in Jinan. Rodents in rural residential areas tested positive for *R. typhi* and *F. tularensis*. *R. typhi* and *A. phagocytophilum* were found positive in only one *M. musculus* and one *S. murinus* from key industries, respectively. These data indicate that the infection rates of these rodent-borne pathogens were low, but it is essential to strengthen rodent control and personal protection in rural areas and key industries.

Although rodents were suspected to be reservoir hosts of bunyaviruses, and a previous study found a pronounced discrepancy in the prevalence of severe fever with thrombocytopenia syndrome virus (SFTSV) in rodents (14), no positive results were found in our study. This finding was generally consistent with the results of a study conducted in Zhejiang Province, which tested 1,604 rodents for SFTSV (7). Scrub typhus is an emerging infectious disease caused by *O. tsutsugamushi*. Among rodents captured in autumn, the *O. tsutsugamushi* infection rate was 8.1%; however, none of the 68 rodents captured during spring, summer, and winter tested positive for *O. tsutsugamushi* (15). The negative results in our research might be due to low infection rates of DBV and *O. tsutsugamushi* or seasonal susceptibility in rodents.

This study has three limitations. First, continuous rodent surveillance was not possible due to changes in the rodent survey program. Second, only a selection of zoonotic pathogens in rodents and shrews were screened, rather than all pathogens, which would have required next-generation sequencing. Third, the sample size of rodents and shrews varied by geographic region and species.

Knowledge of rodent capture rates, seasonality, geographic distribution, and zoonotic pathogens is important for clinical recognition and personal protection. Strengthening ecological and pathogenic surveillance of rodents can facilitate the prediction and early warning of related diseases. Maintaining low capture rates of host animals in rural areas remains a vital measure for preventing rodent-borne diseases in

China.

**Conflicts of interest:** No conflicts of interest.

**Acknowledgements:** The staff for their participants of the Municipal and County Centers for Disease Control and Prevention in Shandong Province.

**Funding:** Supported by Shandong Provincial Natural Science Foundation (ZR202212020225); Youth Innovation Fund of Shandong Center for Disease Control and Prevention (QC-2022-07); Shandong Provincial Traditional Chinese Medicine Science and Technology Project (Q-2023101).

doi: 10.46234/ccdcw2024.193

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Submitted: March 25, 2024; Accepted: August 30, 2024

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## SUPPLEMENTARY MATERIAL

SUPPLEMENTARY TABLE S1. The type of tissue and limit of detection for rodent borne pathogens\*.

Pathogen	Tissue <sup>†</sup>	Detection method	Limit of detection <sup>§</sup>
Hantavirus	Lung	Taqman real-time RT-PCR	Cq≤35
Dabie bandavirus	Liver, Spleen, Kidney	Taqman real-time RT-PCR	Cq≤35
<i>Leptospira interrogans</i>	Liver, Spleen, Kidney	Taqman real-time PCR	Cq≤35
<i>Rickettsia typhi</i>	Liver, Spleen, Kidney	Taqman real-time PCR	Cq≤36
<i>Orientia tsutsugamushi</i>	Liver, Spleen, Kidney	Taqman real-time PCR	Cq≤35
<i>Anaplasma phagocytophilum</i>	Liver, Spleen, Kidney	Taqman real-time PCR	Cq≤33
<i>Francisella tularensis</i>	Liver, Spleen, Kidney	Taqman real-time PCR	Cq≤36

\* This table refers to the National Vector Etiology Surveillance Program (Trial) by Chinese Center for Disease Control and Prevention;

<sup>†</sup> The tissue mixed by homogenate;

<sup>§</sup> When the Cq value is less than or equal to the limit of detection, it is positive, otherwise, it is negative, meanwhile, it needs to be adjusted appropriately according to the amplifiers.



SUPPLEMENTARY TABLE S2. The rodent capture rate, species composition and prevalence of pathogen infection in different regions of Shandong Province.

Rodents	Ji nan	Qing dao	Zi bo	Zao zhuang	Dong ying	Yan tai	Wei fang	Ji ning	Tai an	Wei hai	Ri zhao	Lin yi	De zhou	Liao cheng	Bin zhou	He ze	Total
<i>R. norvegicus</i>	218	259	307	29	161	299	243	44	37	38	19	7	66	111	19	262	2,119
<i>R. flavipectus</i>	0	1	14	0	0	2	3	3	9	0	0	0	0	1	0	1	34
<i>M. musculus</i>	167	274	89	49	159	74	127	43	56	35	71	6	3	185	54	233	1,625
<i>A. agrarius</i>	2	80	14	7	59	1	11	0	4	2	4	5	0	12	1	12	214
Others*	10	34	3	0	14	30	0	6	2	1	4	14	0	12	19	4	153
Effective traps	73,839	69,669	53,678	13,487	19,701	64,395	63,883	23,387	18,450	6,239	19,631	10,435	11,431	70,946	32,619	41,773	593,563
No. of rodents	397	648	427	85	393	406	384	96	108	76	98	32	69	321	93	512	4,145
Capture rates (%)	0.54	0.93	0.8	0.63	1.99	0.63	0.6	0.41	0.59	1.22	0.5	0.31	0.6	0.45	0.29	1.23	0.7
Pathogens	Pathogen detection		Jinan	Qingdao	Zibo	Dongying	Yantai	Weifang	Jining	Tai'an	Wei hai	Linyi	Binzhou	Heze	Total		
<i>Hv</i>	No. rodents		100	200	400	428	104	367	144	400	137	150	115	203	2,748		
	No. positive		0	7	0	2	0	57	4	1	0	0	0	0	71		
	Positive rate (%)		0	3.5	0	0.47	0	15.53	2.78	0.25	0	0	0	0	2.58		
<i>BDV</i>	No. rodents		100	200	400	428	122	367	144	400	137	150	118	203	2,769		
	No. positive		0	0	0	0	0	0	0	0	0	0	0	0	0		
	Positive rate (%)		0	0	0	0	0	0	0	0	0	0	0	0	0		
<i>Leptospira interrogans</i>	No. rodents		100	200	400	428	–	367	144	400	137	150	118	203	2,647		
	No. positive		9	1	0	9	–	5	0	0	0	0	2	3	29		
	Positive rate (%)		9	0.5	0	2.1	–	1.36	0	0	0	0	1.69	1.48	1.1		
<i>Rickettsia typhi</i>	No. rodents		100	0	400	0	–	0	42	400	–	20	–	–	962		
	No. positive		9	0	0	0	–	0	0	0	–	0	–	–	9		
	Positive rate (%)		9	0	0	0	–	0	0	0	–	0	–	–	0.94		
<i>Ot</i>	No. rodents		100	0	400	0	122	0	0	400	–	20	–	–	1,042		
	No. positive		0	0	0	0	0	0	0	0	–	0	–	–	0		
	Positive rate (%)		0	0	0	0	0	0	0	0	–	0	–	–	0		
<i>Anaplasma phagocytophilum</i>	No. rodents		100	0	104	–	–	0	0	400	–	20	–	–	624		
	No. positive		1	0	0	–	–	0	0	0	–	0	–	–	1		
	Positive rate (%)		1	0	0	–	–	0	0	0	–	0	–	–	0.16		
<i>Francisella tularensis</i>	No. rodents		100	0	0	–	–	0	0	400	–	20	–	–	520		
	No. positive		1	0	0	–	–	0	0	0	–	0	–	–	1		
	Positive rate (%)		1	0	0	–	–	0	0	0	–	0	–	–	0.19		

Abbreviation: Hv=Hantavirus; BDV=Dabie bandavirus; Ot=*Orientia tsutsugamushi*.

\*– No testing was conducted.

SUPPLEMENTARY TABLE S3. The positive rate of pathogens in different years.

Pathogen	2020			2021			2022		
	Positive	Test	Positive rate (%)	Positive	Test	Positive rate (%)	Positive	Test	Positive rate (%)
Hantavirus	8	220	3.64	25	1,463	1.71	38	1,065	3.57
Dabie bandaviru	0	220	0	0	1,484	0	0	1,065	0
<i>Leptospira interrogans</i>	14	216	6.48	9	1,366	0.66	6	1,065	0.56
<i>Rickettsia typhi</i>	9	121	7.44	0	399	0	0	442	0
<i>Orientia tsutsugamushi</i>	0	125	0.00	0	517	0	0	400	0
<i>Anaplasma phagocytophilum</i>	1	121	0.83	0	199	0	0	304	0
<i>Francisella tularensis</i>	1	121	0.83	0	199	0	0	200	0

Note: Except for 5 positive cases that were HTNV type in 2022, all the other positive cases were SEOV type.