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SMALL MAMMALS AS RESERVOIRS AND VECTORS OF YERSINIOSIS PATHOGENS (*YERSINIA ENTEROCOLITICA* AND *Y. PSEUDOTUBERCULOSIS*)

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Abstract

Yersinia infections are recorded worldwide and sapronotic natural foci of *Yersinia enterocolitica* and *Y. pseudotuberculosis* infections also occur in the Crimean Peninsula. The distribution and prevalence of pathogenic *Yersiniae* among small mammals of the Crimean Peninsula is analysed based on results of epizootiological monitoring of natural foci infections. Pathogenic *Y. enterocolitica* were found in 10 species of small mammals, and the average number of infected specimens in the Crimea was 0.11 ± 0.03. The highest prevalence of yersiniosis pathogens was recorded among specimens of *M. socialis* (4.22 %), *M. spicilegus* (2.06 %), *C. leucodon* (1.96 %), *S. flavicollis* (1.85 %), and *S. uralensis* (1.33 %). The number of small mammals that are carriers of pathogens of yersinioses varies significantly in different natural zones of the Crimean Peninsula. In the mountain-forest zone, the prevalence of *Y. enterocolitica* among Micromammalia is 2.94 %, in the foothills it decreases to 0.99 %, in the lowland — to 0.77 % with a lowest value of 0.62 % in steppe areas of the Kerch Peninsula. Results show a decreasing pattern of prevalence of *Y. enterocolitica* among small mammals from the mountain-forest zone to plain steppe. A reverse trend was revealed for the prevalence of *Y. pseudotuberculosis* among Micromammalia: 0.03 % in the mountains, 0.17 % in the foothills, and 0.25 % in the steppe. The number of trap-lines with records of Micromammalia having both infections varies from 18.3 % in the foothills to 21.3 % in the mountains and 24.8 % in the steppe zone. The portion of trap-lines with three and more infections is also high (6.7 % in the mountains and foothills and 5.5 % in the steppe). The obtained results show a wide distribution of combined foci in the Crimea. Considering that, in the peninsula, several tick-transmitted and other zoonotic infections (e.g. tick-borne encephalitis and borrelioses, anaplasmosis, ehrlichiosis, Marseilles fever, Q fever, etc.) are widely distributed in the same areas and the pathogens of which are able to reproduce in the same small mammal species as those of yersiniosis and pseudotuberculosis, the real number of combined foci and their diversity in the Crimea could be 3 to 5 times higher.

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SMALL MAMMALS AS RESERVOIRS AND VECTORS OF YERSINIOSIS PATHOGENS (*YERSINIA ENTEROCOLITICA* AND *Y. PSEUDOTUBERCULOSIS*)

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Small mammals as reservoirs and vectors of yersiniosis pathogens (*Yersinia enterocolitica* and *Y. pseudotuberculosis*). — I. Evstafiev. — Yersinia infections are recorded worldwide and sapronotic natural foci of *Yersinia enterocolitica* and *Y. pseudotuberculosis* infections also occur in the Crimean Peninsula. The distribution and prevalence of pathogenic *Yersiniae* among small mammals of the Crimean Peninsula is analysed based on results of epizootiological monitoring of natural foci infections. Pathogenic *Y. enterocolitica* were found in 10 species of small mammals, and the average number of infected specimens in the Crimea was 0.11 ± 0.03. The highest prevalence of yersiniosis pathogens was recorded among specimens of *M. socialis* (4.22 %), *M. spicilegus* (2.06 %), *C. leucodon* (1.96 %), *S. flavicollis* (1.85 %), and *S. uralensis* (1.33 %). The number of small mammals that are carriers of pathogens of yersinioses varies significantly in different natural zones of the Crimean Peninsula. In the mountain-forest zone, the prevalence of *Y. enterocolitica* among Micromammalia is 2.94 %, in the foothills it decreases to 0.99 %, in the lowland — to 0.77 % with a lowest value of 0.62 % in steppe areas of the Kerch Peninsula. Results show a decreasing pattern of prevalence of *Y. enterocolitica* among small mammals from the mountain-forest zone to plain steppe. A reverse trend was revealed for the prevalence of *Y. pseudotuberculosis* among Micromammalia: 0.03 % in the mountains, 0.17 % in the foothills, and 0.25 % in the steppe. The number of trap-lines with records of Micromammalia having both infections varies from 18.3 % in the foothills to 21.3 % in the mountains and 24.8 % in the steppe zone. The portion of trap-lines with three and more infections is also high (6.7 % in the mountains and foothills and 5.5 % in the steppe). The obtained results show a wide distribution of combined foci in the Crimea. Considering that, in the peninsula, several tick-transmitted and other zoonotic infections (e.g. tick-borne encephalitis and borrelioses, anaplasmosis, ehrlichiosis, Marseilles fever, Q fever, etc.) are widely distributed in the same areas and the pathogens of which are able to reproduce in the same small mammal species as those of yersiniosis and pseudotuberculosis, the real number of combined foci and their diversity in the Crimea could be 3 to 5 times higher.

Key words: yersiniosis, pseudotuberculosis, small mammals, prevalence, mixed infections, Crimea.

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Introduction

Small mammals are an integral part of any natural ecosystem. They are associated with the existence and transmission of pathogens of a number of viral, rickettsial, bacterial, and protozoal infections, including of yersinioses (genus *Yersinia*), among the various members of communities.

According to modern views, the genus *Yersinia* comprises 18 species (Genus Yersinia... 2020), and the term "yersiniosis" is often used for two infectious diseases: enteritis caused by *Y. enterocolitica* (Schleifstein and Coleman 1939) and pseudotuberculosis or Far East scarlet-like fever caused by *Y. pseudotuberculosis* (Pfeiffer 1889) (Naktin & Beavis 1999; Smirnov 2004; Euzéby & Aidan 2014).

In human pathology, in addition to these two enteropathogenic yersiniae, there is a third species — the pathogen of plague *Y. pestis* (Lehmann, Neumann, 1896) (Smirnov 2004).

All *Yersinia* species, regardless to ways of transmission to the host's organism, are able to persist in the lymphoid tissue reproducing in macrophages. *Y. pestis, Y. pseudotuberculosis* serotype O1, and *Y. enterocolitica* biotype 1B possess an island of high pathogenicity the functioning of which is associated with iron utilization essential for the expression of virulence.

There are 76 serotypes of *Y. enterocolitica*, of which only 11 cause disease in humans (Epidemiological... 2009). Pathogenic *Y. enterocolitica* are united into biogroup 1B. They have H- and O-antigens. Several strains possess V- and W-antigens of virulence located on the outer membrane. They have specific and cross-reacting antigens that define intraspecific and common for enterobacteria antigenic connections with *Y. pseudotuberculosis, Brucellae, Escherichia, Salmonellae, Shigellae, Klebsiellae*, and others (Naktin & Beavis 1999; Smirnov 2004; Enteritis… 2020).

Yersinia enterocolitica and *Y. pseudotuberculosis* are heterotrophic facultative anaerobic microorganisms with psychrophilic and oligotrophic features. They grow in minimal media and in media of depleted composition.

Yersinioses are recorded worldwide, but in countries of Western and Northern Europe, in the UK, the USA, Canada, Japan, and Russia the most often, while occasionally in Africa, Asia, South America, and Eastern Europe (Aldová & Láznicková 1979; Wang *et al*. 2009; Platt-Samoraj *et al*. 2020).

Material and Methods

The study is based on materials of epizootiological monitoring of natural foci infections collected during field research since 1984 in the Crimean Peninsula. Standard census and trapping methods of small mammals were used (Kucheruk 1952; Kucheruk & Korenberg 1964; Popov 1967; Karaseva & Telitsyna 1996).

In the Crimea, three natural zones are commonly distinguished: steppe, foothills, and mountain-forest zones. The southern coast of the Crimea is not considered here as a separate natural zone, because the real Mediterranean coast is represented only as a narrow belt, which is densely built and practically lacks natural biotopes. Therefore, trappings of small mammals in this area were carried out beyond the settlements in the mountain-forest zone.

Small mammals trapped in natural biotopes were grouped separately by species and trap-lines and were placed into separate canvas bags. Then the collected material was frozen and stored so until dissection, which was carried out in the laboratory of the Department of Especially Dangerous Infections of the Crimean Republican Sanitary-Epidemiological Station.

In the course of dissection, organs of the collected specimens were taken for further serological and bacteriological investigation to reveal pathogens of yersinioses and of other natural foci zoonoses.

Laboratory diagnostics of the field material involved bacteriological, immunological, and serological methods. The main method was bacteriological, when the collected material was first cultivated on storage media and then on solid culture media with subsequent identification. Immunological methods allowed to detect antigens of *Y. enterocolitica*, while serological methods were applied to reveal specific antibodies to antigens of *Y. enterocolitica*. In laboratories of SES, the polymerase chain reaction (PCR) is used to identify pathogens of yersinioses in the collected field material. The growing of cultures and identification of bio- and serotypes was carried out according to generally accepted methods (Epidemiology… 1990; Epidemiological… 2009).

Results

During the census period, 24 250 small mammal specimens were investigated for yersiniosis using bacteriological and serological methods (Table 1), in particular 1 909 specimens of three insectivore species (Soricidae) as well as 22 341 specimens of eight rodent species (Rodentia).

Insectivores are presented by following species: lesser white-toothed shrew (*Crocidura suaveolens*) (1706), bicoloured white-toothed shrew (*Crocidura leucodon*) (153), Eurasian pygmy shrew (*Sorex minutus*) (50). Rodents include five species of the family Muridae — steppe field mouse (*Sylvaemus witherbyi*) (8793), pygmy field mouse (*Sylvaemus uralensis*) (2777), yellow-necked field mouse (*Sylvaemus flavicollis* Melchior, = *tauricus* Pallas) (918), house mouse (*Mus musculus*) (4478), mound-building mouse (*Mus spicilegus*) (534), and two species of the family Arvicolidae — Altai vole (*Microtus obscurus*) (2579), social vole (*Microtus socialis*) (1825), and one species of the family Cricetidae — grey dwarf hamster (*Cricetulus migratorius*) (437).

Table 1. Results of bacteriological and serological investigation of small mammals for *Y. enterocolitica*

Таблиця 1. Результати бактеріологічного і серологічного дослідження дрібних ссавців на *Y. enterocolitica*

Small mammal species	Number of studied specimens	Bacteriology		Serology	
		n (specimens)	Portion* of positives, %	n (specimens)	Portion* of positives, %
C. suaveolens	1706	3	0.012 / 0.176	7	0.029 / 0.410
C. leucodon	153	1	0.004 / 0.654	2	0.008 / 1.307
S. witherbyi	8793	2	0.008 / 0.023	34	0.140 / 0.387
S. uralensis	2777	4	0.016 / 0.144	33	0.136 / 1.188
S. flavicollis	918	4	0.016 / 0.436	13	0.054 / 1.416
M. musculus	4478	3	0.012 / 0.067	40	0.165 / 0.893
M. spicilegus	534	1	0.004 / 0.187	10	0.041 / 1.873
M. obscurus	2579	1	0.004 / 0.039	18	0.074 / 0.698
M. socialis	1825	10	0.041 / 0.548	67	0.276 / 3.671
C. migratorius	437	0	0.000	4	0.016 / 0.915
In total	24250	29	0.120	228	0.940

* Note: portion of positive results (i.e. infected specimens) from the total number of studied Micromammalia / from the number of studied specimens of the species.

Yersinia enterocolitica

Antibodies to *Y. enterocolitica* were found in 10 species, negative results were obtained only for *Sorex minutus* (the portion of this species was only 0.2 % of the number of studied small mammal specimens). Yersiniae were revealed in 29 specimens (0.12 %) of nine species by bacteriological methods and in 228 specimens (0.94 %) of 10 species by serological methods. The average number of Micromammalia infected by *Y. enterocolitica* in the Crimea was 0.11 ± 0.03 specimens (Table 1).

The highest (above average) detection of yersinionis pathogens was found in *M. socialis* (0.32 % of all studied specimens) followed by the mice *M. musculus* (0.18 %), *S. uralensis* (0.15%), and *S. witherbyi* (0.15 %)1.

Analysis of the portion of infected specimens of separate species from the number of studied specimens of this species showed that the pathogen is revealed in 4.22 % of specimens of *M. socialis*, 2.06 % of *M. spicilegus*, 1.96 % of *C. leucodon*, 1.85 % of *S. flavicollis*, and 1.33 % of *S. uralensis.* In the other species, this parameter varies from 0.92 % in *C. migratorius* to the minimum 0.41 % in *S. witherbyi.*

The number of small mammals carrying yersiniosis pathogens significantly differs in various natural zones of the Crimean Peninsula. The analysis shows a decreasing tendency of prevalence of yersiniosis among small mammals from the mountain-forest zone to plain steppe biotopes. The portion of infected animals reaches 2.94 % in the mountain-forest zone, 0.99 % in the foothills, and 0.77 % in the plain steppe with the lowest 0.62 % in steppe areas of the Kerch Peninsula (Fig. 1).

Yersinia pseudotuberculosis

Analysis of serological data on the prevalence of *Y. pseudotuberculosis* among small mammals revealed that this pathogen occurs significantly less often than *Y. enterocolitica*. A total of 51 positive results were obtained: 20 in *M. musculus* (39.2 %), 18 in *S. witherbyi* (35.3 %), 4 in *M. socialis* (7.8 %), 3 in *C. suaveolens* (5.9 %), 2 in both *S. uralensis* and *C. migratorius*, and 1 in both *M. spicilegus* and *M. obscurus*.

When analysing the spatial (zonal) distribution of positive cases in the territory of the peninsula it was found that the highest number of positive results was revealed in steppe areas — 45 (88.2 %), of which 32 (62.8 %) in the lowland part of Crimea and 13 (25.5 %) in the Kerch Peninsula.

1 Here and in the next paragraph: the number of infected animals is the sum of specimens with positive results for *Y. enterocolitica* by both serological and bacteriological methods.

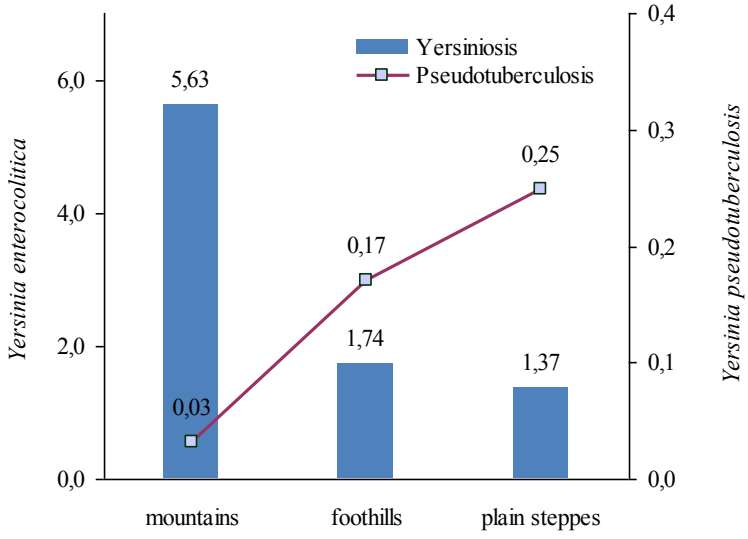

Fig. 1. Distribution of infected small mammals by natural zones of the Crimean Peninsula (% of infected animals from the number of studied specimens in this zone).

Рис. 1. Розподіл заражених дрібних ссавців за природними зонами Кримського півострова (доля заражених мікромамалій у % від числа досліджених особин у цій зоні).

In the mountain-forest zone, only one positive result was obtained, while in the foothills, which have an intermediate location between the steppe and mountain-forest zone, 5 (9.8 %). The portion of infected specimens in steppe areas of the Crimea is 0.24 % in the Kerch Peninsula and 0.25 % in the lowland part of Crimea. In the foothills, the portion of infected specimens is 0.17 %, whereas in the mountain-forest zone is only 0.03 % (Fig. 1).

Discussion

Natural foci of yersinioses

Epizootiological studies that have been carried out in the past decades in the Crimean Peninsula allowed to reveal the role of separate small mammal species as the main structural components of several natural foci ecosystems (Markeshin *et al.* 1991; Alekseev *et al*. 1996; Evstafiev 2001; Tovpinets & Evstafiev 2003; Evstafiev *et al*. 2006 and others). The obtained preliminary data suggest that pathogenic *Y. enterocolitica* occur in the entire areas of the peninsula with a relatively even distribution. As our studies showed, pathogenic *Y. pseudotuberculosis*, and respectively its distribution range, is related to steppe areas of the peninsula and is practically absent in wooded and shrubby biotopes of the mountain-forest zone.

Several studies showed that pathogenic *Y. enterocolitica* are sapronotic organisms having the soil as their natural reservoir (Naktin & Beavis 1999; Smirnov 2004). Yersiniae, as other sapronotic pathogens, usually persist outside living organism thus they are characterised by high resistance to various environmental factors and have a wide range of reaction norm to temperature, moisture, light, etc. Therefore, yersiniae are able to reproduce in a wide range of temperature (from +40 to -3°C) easily adapting to changing conditions, persisting in water for 2 to 8 months and, under favourable conditions, up to a year in the soil (Smirnov 2004; Enteristis… 2020).

On the other hand, due to their facultative psychrophilicity, *Y. enterocolitica* is able to reproduce also in the organism of mammals interacting with the host animals nonspecifically and episodically. Therefore, yersinioses are considered as zoophilic sapronotic infections, when the pathogen exists successfully both in the organism of warm-blooded animals acting as casual parasite and in the environment. As a result, they do not display any specific features regarding hosts and are able to parasite in a large number of animal species.

Since the pathogens of yersiniosis live in the gastrointestinal tract, they are excreted into the environment with faeces, thereby infecting the soil, water and various objects. Infection of other animals can occur alimentary by consuming contaminated food.

Therefore, the faecal-oral mechanism of transmission of the disease is the main one, and the "entrance gate" of the disease is usually the gastrointestinal tract.

Earlier it was shown that practically all types of small mammals of the Crimean mammal fauna, both insectivores and rodents, can be infected with yersiniae, becoming reservoirs (hosts) of pathogens of yersiniosis. Reservoirs of Yersiniae, in addition to wild animals, can also be domestic and farm animals (cats, dogs, pigs, cattle, etc.) (Evstafiev *et al.* 2006; Epidemiological... 2009). Recent studies have shown that Yersiniae and some other pathogenic microorganisms can also infect bats causing their death. (Imnadze *et al.* 2020).

Bats, widespread in Crimea, are of considerable interest from the point of view of epizootiology. Although they are not in close and constant contact with terrestrial small mammals, the exchange of pathogens of various transmissive infections is still possible both directly and through blood-sucking arthropods. The likelihood of such contacts and exchange of pathogens increases due to the long life span of bats and their mobility.

When studying natural foci of yersinioses, it is necessary to consider their main components in the entire set of biocoenotic connections, which contributes to the knowledge of the processes occurring in focal ecosystems.

Combined foci and mixed infections

Many small mammals of the Crimean fauna, especially common dominant species, can be reservoir hosts for several types of pathogens, which manifests itself in the form of mixed infections. At the same time, the components of one ecosystem can be pathogens of both transmissible and a number of non-transmissible infections, the latter including sapronotic and opportunistic microorganisms. Little is known about the nature of interaction of the entire complex of these organisms at different levels of biological organisation. (Korenberg 2000).

Almost the entire territory of the Crimea is a combination of natural foci of various infections. Their individual structural components are simultaneously included in the parasitic systems of several pathogenic and (or) opportunistic microorganisms (Alekseev *et al.* 1996; Grigoryan *et al.* 2001).

The pathogen population is an obligatory and specific component of any natural ecosystem that constitutes the focus of a natural focal disease (Litvin & Korenberg 1999). At the same time, in each carrier and transmitter, a specific parasitocoenosis, or microcommunity, is formed (Balashov 2000) that includes viruses, rickettsia, bacteria, and other microorganisms. Under different landscape and ecological conditions, such microcommunities can differ significantly by the set of microorganisms in their composition.

Usually, in the body of a vertebrate host or an arthropod vector, with rare exceptions, there is no interference between different pathogens. They are often localized in certain specific ecological niches, which are separate organs, tissues, or certain cell structures. This ensures the relative autonomy of parasitic systems and the possibility of the existence of mixed natural foci (Friedhoff 1990; Korenberg 1998, 2001; Balashov 1999).

Interesting are the data on distribution of trap-lines used to capture small mammals in the Crimea depending on the detection of infected animals on them. Here the author presents data on four natural focal zoonotic (tularaemia and leptospirosis) and sapronotic (yersiniosis and pseudotuberculosis) infections (Table 2).

The data in Table 2 show that the portion of trap-lines with small mammals infected with only four types of pathogens is quite high and in total in the Crimea is 11.9 %. This indicates a wide distribution of natural foci of these infections in the peninsula. The maximum number of lines with infected animals is concentrated in the mountain-forest (21.1 %) and foothill (17.3 %) zones; this value is lower in the steppe zone (9.6 %). This may indicate both a wider distribution of the considered infections in the mountain-forest zone and a more aggregated distribution of their micro-foci in steppe areas of the lowland part of the Crimea.

The obtained data (Table 2) also indicate a wide distribution of combined foci in the peninsula.

Table 2. Distribution of trap-lines with infected small mammals (four natural focal infections) in natural zones of the Crimean Peninsula

Таблиця 2. Розподіл облікових ліній з інфікованими Micromammalia (чотирма природно-вогнищевими інфекціям) по природним зонам Кримського півострова

Parameter	Mountains	Foothills	Steppe	In total
Number of trap-lines *)	355/12.41	346/12.10	2149/75.14	2860
Number of trap-lines with infections **)	75/21.13	60/17.34	206/9.59	341/11.92
Number of trap-lines with 1 infection ***)	54/72.00	45/75.00	1445/70.39	244/71.55
Number of trap-lines with 2 infections ***)	16/21.33	11/18.33	51/24.76	78/22.87
Number of trap-lines with 3 infections ***)	5/6.67	4/6.67	8/3.88	17/4.99
Number of trap-lines with 4 and 5 infections ***)	–	–	1/0.49	1/0.29

Note: in the numerator — the number of trap-lines; in the denominator — *) portion (%) from the total number of trap-lines; **) portion (%) from the total number of trap-lines exposed in this zone; ***) portion (%) from the total number of trap-lines with infected small mammals in this zone.

The number of lines on which small mammal were found with two infections ranges from 18.3 % in the foothills to 21.3 % in the mountains and 24.8 % in the steppe zone. The portion of lines with three or more infections is quite high (6.7 % in the mountains and foothills, and 5.5 % in the steppe).

Previous studies of natural foci of zoonotic infections showed their wide variety in the Crimea (Evstafiev 2001; Tovpinets & Evstafiev 2003; Evstafiev *et al.* 2006, and others). In the forests in the south of the peninsula, such vector-borne infections as Lyme borreliosis, tick-borne encephalitis, granulocytic anaplasmosis, human monocytic ehrlichiosis, Marseilles fever and others are widespread. Since they are not considered in this article, in reality, the number of trap-lines on which animals infected with various natural focal infections were caught is much larger.

The spread of any kind of mixed natural foci is determined mainly by the degree of sympatry of the geographic ranges of the corresponding pathogens and the specifics of their requirements to abiotic and biotic environmental factors. Naturally, on the periphery or in the pessimal parts of the range, a certain pathogen exists only in the most favourable ecosystems, and the type of mixed focus, common for most of the area of its sympatry with another pathogen, is rare or absent here.

Conclusions

In the territory of the Crimean Peninsula, there are sapronotic natural foci of intestinal yersineosis *Yersinia enterocolitica* and pseudotuberculosis *Y. pseudotuberculosis*. Pathogenic *Y. enterocolitica* were found in 10 species of small mammals and the average number of infected individuals in the Crimea is 0.11 ± 0.03.

The highest prevalence of yersinioses pathogens was noted in *M. socialis* (4.22 %), *M. spicilegus* (2.06 %), *C. leucodon* (1.96 %), *S. flavicollis* (1.85 %), and *S. uralensis* (1.33 %).

Significant differences were revealed in the number of small mammals infected by pathogens of yersiniosis and pseudotuberculosis in various natural zones. The prevalence of intestinal yersiniosis among small mammals was 2.9 % in the mountain-forest zone, 1.0 % in the foothills, and 0.8 % in the steppe zone. The prevalence of pseudotuberculosis demonstrates a reverse trend: 0.03 % in the mountains, 0.17 % in the foothills, and 0.25 % in the steppe.

It was found that combined foci of both zoonotic and sapronotic infections occur in the entire territory of the Crimean Peninsula, as a result of which they manifest themselves in a more or less pronounced form of mixed infections in infected objects (both animals and humans).

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