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Abstract

Nosemosis is the most common disease in honey bee *Apis mellifera* L., and is a major issue related to bee health worldwide. Therefore, the purpose of this research study was to determine prevalence of microsporidia parasitic infection of the genus *Nosema* spp. in East Kazakhstan Region (EKR). In the years of 2018 – 2021, 394 honey bee samples were collected at 30 apiaries located in four districts of East Kazakhstan Region (Katon-Karagay, Urzhar, Borodulikhinsky, and Shemonaikhinsky). In order to determine the level of infestation, firstly, the presence of *Nosema* spp. spores was detected using optical microscopy, and then the average amount of spores per bee was counted using a hemocytometer. The degree of nosemosis prevalence was determined in points by means of a semi-quantitative method, and as a percentage from the total of samples and of the amount of positive tests. At the outcome of the study, microsporidia of the genus *Nosema* spp. were detected in 23.3% of cases (92 samples). Prevalence at its low degree was found in 6 samples (1.5%), at an average degree in 55 samples (14%), and at a high one in 31 samples (7.9%). This research study proved that microsporidia of the genus *Nosema* spp. are widely spread at the apiaries of East Kazakhstan Region in different orographic and climatic conditions. Notwithstanding that it was impossible to statistically

76 determine any significant differences between the dependence of nosemosis prevalence and the
77 apiary location, this indicator is actually higher in the mountainous regions than in the steppe.
78 Concurrently, a close inverse correlation was recognized between the amount of spores in one
79 bee and the level of infestation in bee families from the duration of the vegetation season at the
80 apiary location. This gives grounds to assert that the environmental factors have an impact on
81 formation and development of nosemosis. The results of the research presented in the article
82 indicate the need for further research aimed at increasing the number of studied apiaries, and
83 above all the use of molecular biology methods to distinguish the species that cause nosemosis
84 infection (PCR).
85

86 ## Introduction

87 Honey bee is the most important insect-pollinator of agricultural crops worldwide, pollinating
88 more than 90% of all flowering plants, and is of particular scientific and practical interest as a
89 resource species (Food and Agriculture Organization of the United Nations (FAO), 2006; Botías
90 et al., 2013; Kucher et al., 2016; Chen et al., 2013; Cridland et al., 2017; Danner et al., 2017;
91 Doublet et al., 2017; Ostroverkhova, 2018; Rodriguez García et al., 2018). Besides, honey bee is
92 an asset of economic value owing to the selection of beekeeping products used by people for
93 nutrition and medical treatment (Sforcin et al., 2017; Pasupuleti et al., 2017; Shumkova et al.,
94 2018). The study of the honey bee *Apis mellifera* L. (Hymenoptera: Apidae) being an
95 ecologically and economically significant species, represents a topical and essential research
96 study worldwide (Krivtsov and Lebedev, 2011; Kucher et al., 2016; Tlak Gajger et al., 2015;
97 Ostroverkhova, 2018).
98 Production of honey and beekeeping products in East Kazakhstan Region is a long-standing
99 tradition based on diverse and rich vegetation suitable for honey production, as well as because
100 of the presence of favourable natural, climatic and ecological conditions. East Kazakhstan
101 Region is not only the birthplace of apiculture for Kazakhstan, but is also the main exporter of
102 honey in the Republic (Rib, 2004; Mikheeva, 2016).
103 It is known that various pathogens and honey bee parasites produce an adverse impact on bee
104 families' lifespan. Additionally, they are considered to be among the key factors causing global
105 deaths of honey bee families (Murilhas, 2002; Higes et al., 2010; Evans и Schwarz, 2011; Botías
106 et al., 2013; Tlak Gajger et al., 2015; Doublet et al., 2017; Higes et al., 2020; MacInnis et al.,
107 2021; Marín-García et al., 2022).
108 Some of the most dangerous and widespread parasites on adult honey bees are microsporidia of
109 the genus *Nosema* spp. (Nosematidae), parasitizing in the epithelial cells of the mid-gut (Bailey,
110 1981; Matheson, 1993; Chernyshev, 2012; Rangel et al., 2015; Golubeva, 2018; Jabal-Uriel et
111 al., 2022). To date, there have been identified several species of microsporidia that affect honey
112 bee, i.e. *Nosema apis* (Zander, 1909) and *Nosema ceranae* (Fries et al., 1996). Clinical
113 manifestations of *Nosema* spp. infection vary by pathogen, at individual and colonial levels
114 alike. For instance, an entirely different epidemiology attributes to *Nosema ceranae*, and
115 consistently occurs at the hives throughout the year, which, therefore, leads to reduction and
116 destruction of the colony. On the contrary, *Nosema apis* takes a seasonal pattern with a typical
117 peak of its occurrence and effect on bee families in winter, causing diarrhoea, the presence of

118 which is noticeable by way of defecated hive frames, honeycombs, outer walls of the hive, etc.
119 (Higes et al., 2005; Paxton et al., 2007; Il'yasov et al., 2013; Dar et al., 2013; Zinatullina, 2016;
120 Zinatullina et al., 2017; Doublet et al., 2017; Tokarev et al., 2018; Fleites-Ayil et al., 2018; Li
121 et al., 2019; Higes et al., 2020).
122 Unlike *Nosema apis*, the large-scale pathogenic effect of *Nosema ceranae* on the honey bee
123 body system was recorded (Martín -Hernández et al., 2007; Higes et al., 2010; Golubeva, 2018).
124 The conclusions were drawn on localization of the *Nosema ceranae* spores in various organs and
125 tissues of honey bee, for instance, in malpighian tubule system, fat body, hypopharyngeal and
126 salivary glands, etc. (Chen et al., 2009; Gisder et al., 2010; Golubeva, 2018). However, there is
127 recent evidence of high tropism in two species of *Nosema* spp. to the cells of ventricular
128 epithelium. Thus, both of these microsporidia can negatively affect the life expectancy of
129 individuals in a bee colony, disrupting age-related polyethism with an intensity reflecting their
130 prevalence in the colony (Higes et al., 2020).
131 Very recently, a new parasite, *Nosema neumanni* (Chemurot et al., 2017), was found in the
132 Ugandan bees of, thus far, unknown occurrence and undescribed impact on *A.mellifera* L.
133 (Giovanni et al., 2018; Mazur et al., 2022).
134 *Nosema apis* and *Nosema ceranae* were found on all continents where *A. mellifera* L. is present.
135 For instance, in Africa (Fries et al., 2003; Higes et al., 2009), in Europe (Higes et al., 2006;
136 Paxton et al., 2007; Zinatullina et al., 2011, 2018; Chernyshev, 2012; Golubeva, 2018;
137 Ostroverkhova, 2020), in the Americas (Chen et al., 2008; Calderón et al., 2008), and in Asia
138 (Chen et al., 2009; Shirzadi et al., 2021).
139 The first case of infestation of bees by microsporidia of the genus *Nosema* spp. in Kazakhstan
140 was recorded in 2012 in Ulan District (the village of Saratovka) of East Kazakhstan Region
141 (Ospanova et al., 2012), where a number of apiaries died out, as well as in Katon-Karagay
142 Region in 2015 (Valitova, 2017). Moreover, it was possible to witness an acute type of
143 nosemosis: the infected bees died in a short span of time, and the defecated inner walls of the
144 hive were observed, as well as the death of queens, a great amount of the dead bees nearby the
145 entrances and at the bottom of the hive. Owing to the studies carried out by means of scanning
146 electron and light microscopy, the oval, well-translucent spores of an intracellular parasite of the
147 genus *Nosema spp.* were detected in the mid-guts of bees (Ospanova et al., 2012; Valitova,
148 2017). Still, due to the lack of appropriate methods to date of the research study, the diagnostic
149 analysis of the pathogen species did noit take place.
150 It does not seem possible to provide an impartial assessment of the epizootic situation as regards
151 nosemosis, since systematic monitoring of this disease in East Kazakhstan Region did not take
152 place.
153 In light of the fact, that many studies prove negative effects of nosemosis at the level of a bee
154 family (Farrar, 1947; Fries, 1998; Anderson и Giacon, 1992; Higes et al., 2009; Mayack и Naug,
155 2009; Botías et al., 2013; Tlak Gajger et al., 2015; Ostroverkhova, 2018; Golubeva, 2018;
156 Baygazanov and Tikhomirova, 2019, 2020; Kartal et al., 2021; Salkova et al., 2022; Houdelet et
157 al., 2022), the need arises to proceed with monitoring studies of honey bee at the apiaries of East
158 Kazakhstan Region.

159 The relevance of research into microsporidian parasitic infections in honey bee is associated
160 with the development trend in beekeeping in the Republic of Kazakhstan, where, as of 2020, the
161 total of bee families reached 133672 (Bureau of National Statistics of the Agency for Strategic
162 Planning and Reforms of the Republic of Kazakhstan). Of them, 71% or 94421 bee families are
163 concentrated in East Kazakhstan Region and produce up to 80% of the total honey of the
164 Republic annually.
165   In connection therewith, the purpose of this research was to study infestation of honey
166 bee by microsporidia of the genus *Nosema* spp. at the apiaries of different districts in East
167 Kazakhstan Region. Species-level identification of Nosema spp. did not take place at that stage.
168

Materials & Methods
Research sites
171   The research was carried out at Agrotechnopark Scientific Center, at Veterinary and Food
172 Safety Laboratory of Shakarim State University of Semey. The apiaries of East Kazakhstan
173 Region of the Republic of Kazakhstan were used for sampling.
174 The area of sampling and sample testing is located in the northeastern and southern parts of the
175 Region. The lowlands and generally flat plains are typical for the Districts of Borodulikhinsky
176 and Shemonaikhinsky, while Katon-Karagay and Urzhar Districts are located in the mountains.
177 The climate of the studied districts of East Kazakhstan Region is distinctly continental, defined
178 by long severe winters and dry, rather hot and torrid summers, with large temperature
179 fluctuations both according to the year seasons and during the day. Continentality diminishes
180 slightly in the mountainous areas and at the foothills.
181 The climatic conditions of the lowlands and mountainous areas differ from each other in terms of
182 both temperature and amount of precipitation (Shcherbakov et al., 1991; Rieb, 2004; Nurtazin,
183 2017; Natural resources. Area study of Eastern Kazakhstan; Weather Spark. Weather all year
184 round anywhere in the World).
185 In Katon-Karagay Region is Winters are long and relatively cold. Cool and short summers (90 to
186 100 days) precondition brevity of the vegetation season.
187 The climate in Urzhar Region is defined as semi-dry, mildly hot.
188 Borodulikhinsky District is located in the dry steppe subzone of the steppe zone.
189 Shemonaikhinsky District belongs to the mountain meadow steppe and foothill steppe, i.e.
190 moderately humid natural and climatic zones.
191 The vegetation season in Katon-Karagay, Borodulikhinsky, Shemonaikhinsky and Urzhar
192 Districts lasts 100, 122, 126 and 166 days respectively.
193 Figure 1 (A, B) shows the research sites.
194

Sampling
196 The samples (*A. mellifera* L.) were collected from 394 bee colonies from 30 apiaries located in
197 four districts of East Kazakhstan Region (Katon-Karagay, Urzhar, Borodulikhinsky,
198 Shemonaikhinsky) in the spring (April-May) from 2018 to 2021.

199   For the purposes of this research, adult living bees were selected (in the amount of at least 10%
200   of the total of bee families at each apiary), as well as the dead bees were collected near the hives.
201   The samples were taken randomly at the hive entrance or on the hive frames away from the
202   brood nest. Each sample contained at least 50 units for the test study. Samples of the living bees
203   were dusted down into a plastic envelope with free access for air, and into paper bags. The dead
204   bees was collected into separate paper bags. Each sample was numbered. The bag was marked
205   with the beehive number, sample number, and the date of sampling.
206   Prior to laboratory testing, live bees were frozen for immobilization at -20°C for 15-20 minutes
207   by following the protocol set for Nosemosis diagnose in various studies (Antonov et al., 1987;
208   Grobov et al., 1987; Topolska I Hartwig, 2005; Ilyasov et al., 2013; Pohorecka et al., 2018;
209   Shumkova et al., 2018).
210
211   **Research methods**
212   The analysis of infestation of honey bees (*A. mellifera* L.) with microsporidia was carried out in
213   accordance with the "Methodological guidelines for laboratory studies on honey bee
214   nosematosis" in force on the territory of the Republic of Kazakhstan, approved on April 25, 1985
215   (Antonov et al., 1987).
216   Microscopic counting of the bees infected with the causative agents of nosemosis was done by
217   means of a Micmed-5 XC1450 optical microscope as well as a hemocytometer. The
218   hemocytometer counted the amount of nosema spores and was used to count the average amount
219   of spores per bee (Zinatullina, 2018).
220
221   **Method of optical microscopy**
222   The bee samples were tested by a group method. In order to accomplish this, the mid-gut was
223   removed from the frozen units, and the abdomen was taken from the dead bees. Those were then
224   placed in a porcelain mortar with a pestle; distilled water was added at the rate of 1 ml per bee
225   tested. The mortar contents were thoroughly pulverized until they became streak free. A drop of
226   the prepared suspension was applied to the slide plate, and then covered with a cover glass so
227   that no air bubbles remained. Excess water was removed by filter paper. Further-on, microscopy
228   was conducted at a mean magnification (x400) in a slightly darkened field of vision. At least 20
229   fields of vision were viewed (Grobov et al., 1987; Voronin et al., 1974).
230   In the case of a positive test, in the microscope field of vision there were detected oval, slightly
231   curved, sometimes straight or rice-grain shaped spores of the genus *Nosema* spp., strongly
232   refracting the light and with the dimensions of 4.5-7.5x2.0-3.5 µm as shown in Figure 2 (Zander
233   and Böttcher, 1984; Fries et al., 1996).
234
235   **Method of counting the amount of spores and estimation of the level of**
236   **infestation**
237   In order to proceed with quantitative diagnostics of nosemosis, the spores were counted in the
238   hemocytometer of the Goryaev counting-chamber device. The amount of nosema spores was

239   counted in 5 large squares divided diagonally into 16 small hemocytometer grids. Then, by
240   multiplying by 50.000, it was possible to obtain the amount of spores in 1 ml of suspension, or
241   per bee. The level of infestation was assessed on a 4-point scale in view of the amount of spores
242   per bee (Table 1) (Zinatullina et al., 2018; Zinatullina, 2018).
243
244   **Statistical method**
245   Systematization of the source data, statistical processing and analysis of the results were
246   accomplished by means of Microsoft Office Excel 2016 spreadsheets. Statistical analyses were
247   also performed using the online calculators available at https://medstatistic.ru (Marapov, 2013).
248   Reliability of the difference in the obtained data was determined by the Student's t-test. Analysis
249   of the total of apiaries and bee families affected by nosemosis and the degree of prevalence by
250   area was defined using the Student's t-test method when comparing the relative values.
251   Statistical estimates of the extent of bee family infestation and the amount of spores per bee were
252   done using the Student's t-test method when comparing the mean values for independent
253   variables (Tokarev et al., 2018). For this reason, the following key indicators were calculated:
254   mean value, variance, quadratic deviation, standard error, t-test, and significance point.
255   Correlation ratio (r) was calculated in order to define dependence between the amount of spores
256   per bee and the degree of impact upon bee families from the duration of the vegetation season.
257

258   **Results**
259   **Analysis of microscopic examination**
260   For the purposes of determination of prevalence of nosemosis in East Kazakhstan region, the
261   presence of the *Nosema* spp. spores was initially detected in the bee samples employing the light
262   microscopy, and then a haemocytometer was used to count the number of microsporidia per bee.
263   Species-level identification of *Nosema* spp. did not take place at that stage.
264   Microscopic analysis indicated that nosemosis was widespread at the apiaries of
265   East Kazakhstan Region. Out of 30 apiaries under study, with the total of 3692 bee families,
266   nosemosis was detected at 21 apiaries (70%) (Table 2).
267

268   According to results (Table 2), it is concluded that nosemosis infestation was higher in apiaries
269   of mountainous regions (Katon-Karagay and Urzhar) as compared to the regions with steppe
270   slope (Borodulikhinsky and Shemonaikhinsky). In the mountainous regions, infestation of the
271   apiaries by nosemosis reached 83.3 and 80% (81.65% on average), and in the steppe regions
272   62.5 and 50% (56.25% on average). Still the comparative analysis given in Figure 3 shows that
273   there are no statistically significant differences between the amount (proportion) of the apiaries
274   affected by nosemosis and their location (p>0.05).
275

276   **Levels of infestation of bee families**
277   Out of 394 honey bee samples under study, the spores of the microsporidia genus *Nosema* spp.
278   were found in 92 samples, or in 23.3% of the cases.

279 At that, different levels of infestation of honey bee were outlined (in percentage of the total
280 amount of samples/the amount of positive tests). As for the regional average, a low degree of
281 prevalence was detected in 6 samples (1.5/6.5%), average in 55 samples (14.0/59.8%), and a
282 high one in 31 samples (7.9/33.7%).
283 Consequently, infestation of bee families in different districts of East Kazakhstan Region ranged
284 from weak (1 point) to strong (3 points). There was no particularly severe infestation (4 points)
285 of the bees (Table 3).
286
287 Low level of infestation was not detected among bee families of district Katon-Karagay district
288 according to the results given in Table 3. A high degree of prevalence (3 points) was recorded
289 for Katon-Karagay (60% of positive tests) and Borodulikhinsky (52.2%) Districts. In Urzhar and
290 Shemonaikhinsky Districts, the average degree of prevalence prevails (70.0 and 73.7%
291 respectively out of the positive tests).
292 When comparing the two districts (Figure 4), difference in the incidence of bee families with
293 nosemosis remains statistically unreliable (p>0.05).
294
295 In Katon-Karagay and Borodulikhinsky Districts, the amount of spores per bee is significantly
296 higher than in Urzhar (f=48, t=2.56, p=0.011; f=27, t=2.74, p=0.006) and Shemonaikhinsky
297 (f=61, t=2.436, p=0.015; f=40, t=2.6, p=0.009).
298 The statistically significant differences were recorded with regard to the amount of spores per
299 bee versus the duration of the vegetation season in the area (p> 0.05). The vegetation season in
300 Katon-Karagay, Borodulikhinsky, Shemonaikhinsky and Urzhar Districts lasts 100, 122, 126
301 and 166 days, and the amount of spores per bee is 22.6±2.76, 20.9±2.17, 13.5±1.85 and
302 14.83±1.25 million units respectively (Figure 5). The correlation coefficient (r) is -0.720. The
303 relationship between the studied criteria is inverse. The tightness (strength) according to the
304 Cheddock's scale is strong.
305 Consequently, it is possible to ascertain reliably that with reduction in the duration of the
306 vegetation season, the amount of spores per bee and the degree of incidence of nosemosis in bee
307 families increases.
308

309 **Discussion**
310 The outcome of our research studies proved the presence of microsporidia of the genus *Nosema*
311 spp. in 23.3% of the analysed bee samples taken from four districts of East Kazakhstan region.
312   The highest degree of prevalence was recorded in the Katon-Karagay District. Of 31
313 samples tested, 10 (32.2%) were positive (Table 2).
314   In the Urzhar District positive samples of *Nosema* spp. were found in 40 out of 170
315 samples under study (23%).
316   The lowest level of infestation was recorded in the samples of honey bee in the
317 Shemonaikhinsky District. Out of 104 studied samples, only 19 (18.3%) revealed microsporidia
318 of the genus *Nosema* spp. Nonetheless, in Borodulikhinsky District of the same part of the
319 Region, the level of infestation reached 25.8%.

320   Our data show that this type of infection is widespread at the apiaries. Our findings align
321 with the data available in other countries. ~~The research study results as obtained by Authors of~~
322 ~~this research paper align with the data available in other countries.~~ For instance, the analysis of
323 the study results of the bee families representing 18 regions of Russia (Arkhangelsk, Belgorod,
324 Voronezh, Kirov, Leningrad, Moscow, Orenburg, Penza, Tomsk, Tula, Tyumen, Altai Republic,
325 Krasnodar, Perm and Stavropol Territories, Republics of Mari El, Tatarstan, Udmurtia) shows
326 that the apiaries in question are affected by nosemosis (Zinatullina et al., 2018).
327   The studies that took place in a number of European countries inclusive of Ukraine, Spain,
328 France, Germany, Switzerland, Denmark, Finland, Greece, Hungary, the Netherlands, the United
329 Kingdom, Italy, Serbia, Poland, Slovenia, Bosnia and Herzegovina, Sweden, etc. (Zinatullina et
330 al., 2018) endorse the distribution of nosemosis in these countries.
331   In recent times, only *Nosema cerana* has been detected in many countries. For instance, in
332 the course of the survey research in Hatay province it was found that, in overall, 20% of the total
333 count of hives were infected. At that, no *Nosema apis* has been detected (Aykut et al., 2022).
334   The research studies conducted by many scientists proved that microsporidia parasitic
335 infection is predetermined by a number of factors, inclusive of temperature, seasonal patterns,
336 nutrient availability, presence of micro-organisms or absence thereof, as well as the stage of
337 development, sex and genetics of the bearer (resistance to microsporidia), etc. (Willis et al.,
338 2022).
339   It is evident, that the biology of *Nosema* spp. depends on temperature. In fact, development
340 of *Nosema apis* demonstrates a seasonal pattern, in which the level of infestation reaches its peak
341 in spring, decreases during the summer time, and is followed by a repeated lower peak in
342 autumn, after which it again decreases in winter (Traver et al., 2012). At that, *Nosema ceranae*
343 can be detected all year round, it develops more rigorously, particularly in the time frame from
344 April to June. In the recent times, this species of *Nosema* spp. occurs more frequently, and
345 infestation of bee families with it is always higher than that with *Nosema apis* (Traver et al.,
346 2012; Higes et al., 2013; Aykut et al., 2022). This may be owing to the fact that *Nosema ceranae*
347 adapts to temperature fluctuations better (Martín-Hernández et al., 2009). Differences in the
348 number of causative agents as observed in between spring and autumn may equally be related to
349 the size of a bee family, as well as physiological features of bees in these seasons. Older bees
350 have a higher level of contamination with *Nosema ceranae* (Jabal-Uriel et al., 2022).
351   The level of infestation in bee families at the same apiary over the summer time may differ
352 not least because of the field bees from different bee families choosing different plants. Chemical
353 composition of honey and pollen collected from different plants may vary (Jabal-Uriel et al.,
354 2022). Consequently, their impact on bee health will be different as well. For instance, high-
355 quality and diverse pollen nutrition improves the survival rate of the healthy bees and the bees
356 infected with *Nosema ceranae*, whilst the quality of pollen (as reflected through protein content
357 and antioxidative activity) highly impacts the exposure of bees to infection (Martín-Hernández et
358 al., 2018; Jabal-Uriel et al., 2022). The pollen and honey of some plants (manuka) may trigger
359 premature death of bee families (Malone et al., 2001).
360   Combined infection and bee pests entail weakened immunity and, most often, result in
361 more severe infestation of the bee families with nosemosis, as well as their premature death
362 (Martín-Hernández et al., 2018).
363   Occurrence of nosemosis is also impacted by the methods of beekeeping, beekeeper
364 competence, and compliance with sanitary measures at the apiary (Martín-Hernández et al.,
365 2018). In fact, the burden of disease at the apiaries employing the same beekeeping tools for

different hives is significantly higher than that at the apiaries that do not employ the same tools for different hives (Aykut Zerek et al., 2022). When it comes to isolating the queen and then replacing her with a younger one, the proportion of bees infected with nosemosis gets reduced, maintaining the overall infestation at the level compatible with viability of the colony (Martín-Hernández et al., 2018).

Some authors proved, that the endemic bees get infested with nosemosis not as often as the introduced bee races and breeds (Aykut Zerek et al., 2022).

Besides, it is known, that occurrence of nosemosis is often driven by the conditions related to the territorial distribution of bee families.

Adverse environmental conditions (polluted environment, use of pesticide) at the beekeeping sites cause a more severe disease and significantly higher rates of mortality in bees (Martín-Hernández et al., 2018).

There is also a point of view that warm climatic conditions are favourable for distribution of *Nosema ceranae* while *Nosema apis* develops in colder climates (Gisder et al., 2010; Higes et al., 2013; Shumkova et al., 2018).

In the course of the study of 28 samples of *A. mellifera* L., typical for three natural and geographical zones of Bulgaria (Southern, Northern and Western Bulgaria), it was found that the highest level of infestation (77.2%) by *Nosema ceranae* was attributable to the bees from the northern part of the country. Still for the bees from the mountainous regions (the Rhodopes, Southern Bulgaria) it was only 13.9% (Shumkova et al., 2018, 2020).

This correlates well with other results confirming that the longer the cold period lasts, the more likely it is for nosemosis to develop, and the greater the degree of infestation in bee families with microsporidia (Chen et al., 2012; Pacini A. et al., 2021). Given, that duration of the period with the average monthly air temperature below zero is longer in the northern than in the southern regions, the level of infestation with nosemosis can be respectively higher.

The outcome of our research studies showed, that in Katon-Karagay District, located in the northeastern part of East Kazakhstan Region in the mountainous area (the vegetation season does not exceed 100 days), with regard to all considered prevalence rates, there were recorded the highest values of nosemosis in the District, namely 83.3% of all the apiaries under study. In 60% of cases, a high level of infestation of bee families was recorded (3 points), as well as the greatest amount of spores per bee (22.9 million).

In Urzhar Region (mountain terrain, located much to the south of Katon-Karagay Region, with the vegetation season lasting 166 days), the average level of infestation of bee families prevails (2 points in 70% of the cases) at 80% of the apiaries affected by nosemosis, and the amount of spores per bee reaches 14.83 million. This is respectively only 1.33% higher and 6.07% lower than at the apiaries located in the lowlands.

In the steppe (Borodulikhinsky and Shemonaikhinsky Districts), where the vegetation season lasts 122 and 126 days, the climatic conditions are similar, still the rates of occurrence differ. In Borodulikhinsky District, the total of the apiaries and bee families affected by nosemosis, a high level of infestation of bee families and the amount of spores per bee are 12.2% and 7.5%, 31.1%, and respectively 35.4% higher than those in Shemonaikhinsky district are.

Notwithstanding that, it was impossible to determine statistically significant differences between dependence of prevalence of nosemosis and the apiary location, it is crucial to pay attention to the fact that in reality this value is higher in the mountainous areas than in the steppe. This is confirmed by other authors as well (Dar et al., 2013).

411   In addition, our research studies reliably prove, that the amount of spores per bee depends
412   on the duration of the vegetation season: the shorter the vegetation season is, the more spores per
413   bee are, and, consequently, the higher is the level of infestation of bees by nosemosis.
414   As per the authors of this research paper, the orographic factors, geographical zone and
415   duration of the vegetation season, defining the climate and flora of the area, predetermine the
416   quantity and quality of feed, temperature, humidity and other conditions related to existence of
417   the bees. Consequently, independently or together with with other factors, they impact
418   infestation of bees with microsporidia, and are important markers to diagnose and forecast the
419   distribution of nosemosis in any particular district. Nonetheless, in order to support these
420   findings, it is required to have additional data collected under various climatic conditions and
421   implementing different methods of beekeeping.
422

Conclusions

424   Thus, the study results given in this research paper prove the distribution of the causative agent
425   of nosemosis in honey bee in East Kazakhstan Region. Predominantly, there is an average and
426   high level of infestation recorded in bee families.
427   The total of apiaries affected by nosemosis in the mountainous areas is higher than in the
428   lowlands. Still, there are no statistically significant differences with regard to occurrence of
429   nosemosis and the apiary location.
430   Proportion of bee families affected by nosemosis at the apiaries also does not significantly
431   depend on the natural and climatic conditions, or the orographic effects. Nonetheless, a close
432   inverse correlation between the amount of spores per bee and the level of infestation of bee
433   families with the duration of the vegetation season at the apiary location was defined. This gives
434   grounds to assert that the environmental factors have an impact on formation and development of
435   nosemosis.
436   The results of the research presented in the article indicate the need for further research aimed at
437   increasing the number of studied apiaries, and above all the use of molecular biology methods to
438   distinguish the species that cause nasal infection (PCR).
439

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444

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Figure 1

A. Map of Kazakhstan and location of EKR. B. Map of sampling sites in EKR. The ratio of the total of samples/amount of samples infected with microsporidia of the genus *Nosema spp.* is given in brackets

Map data © 2022 Google

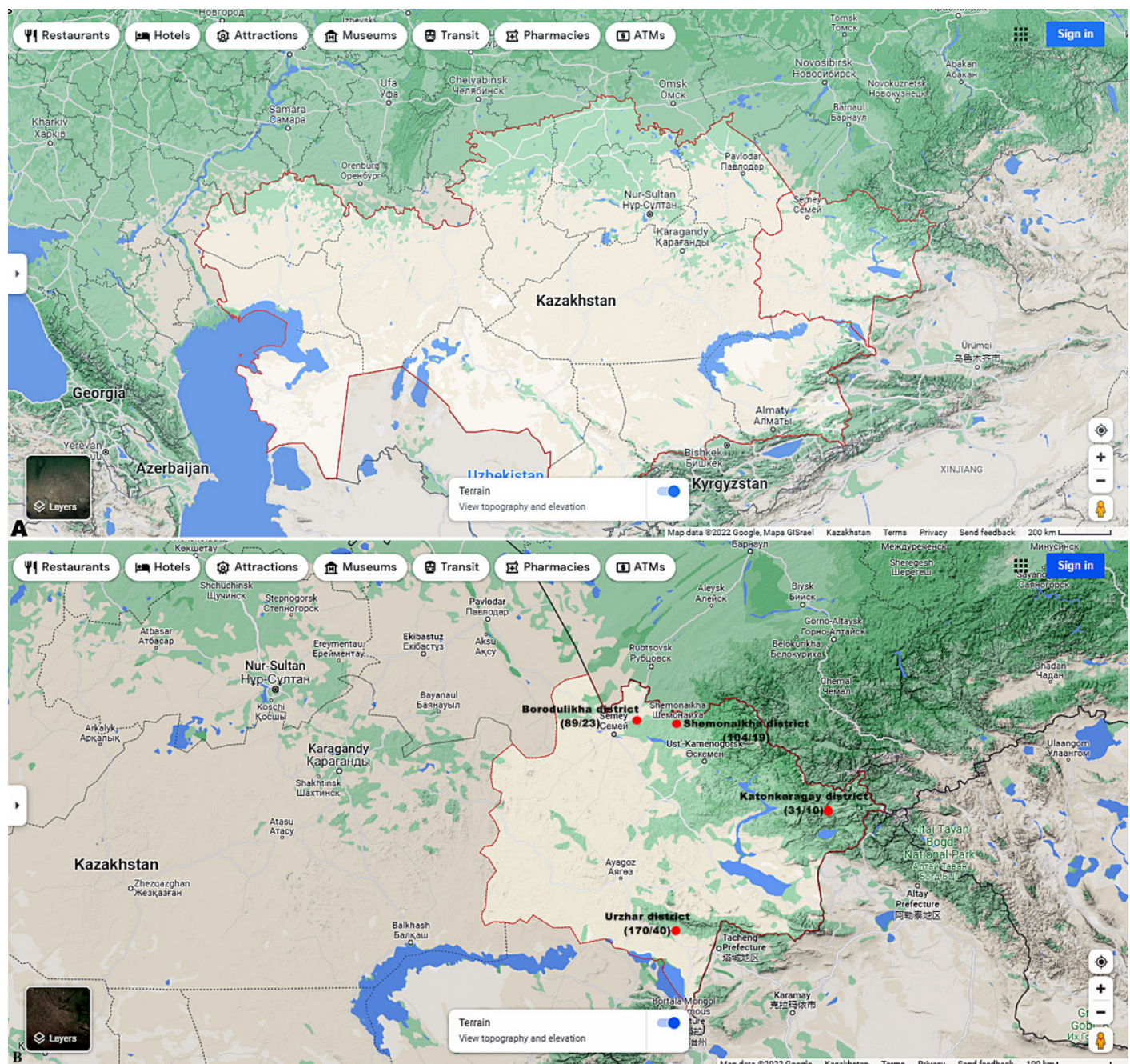



Figure 2

Spores of microsporidia of the genus *Nosema spp.* (x400). The level of infestation of honey bee: a high level of infestation (3 points) is shown in A; a low level of infestation (1 point) is shown in B

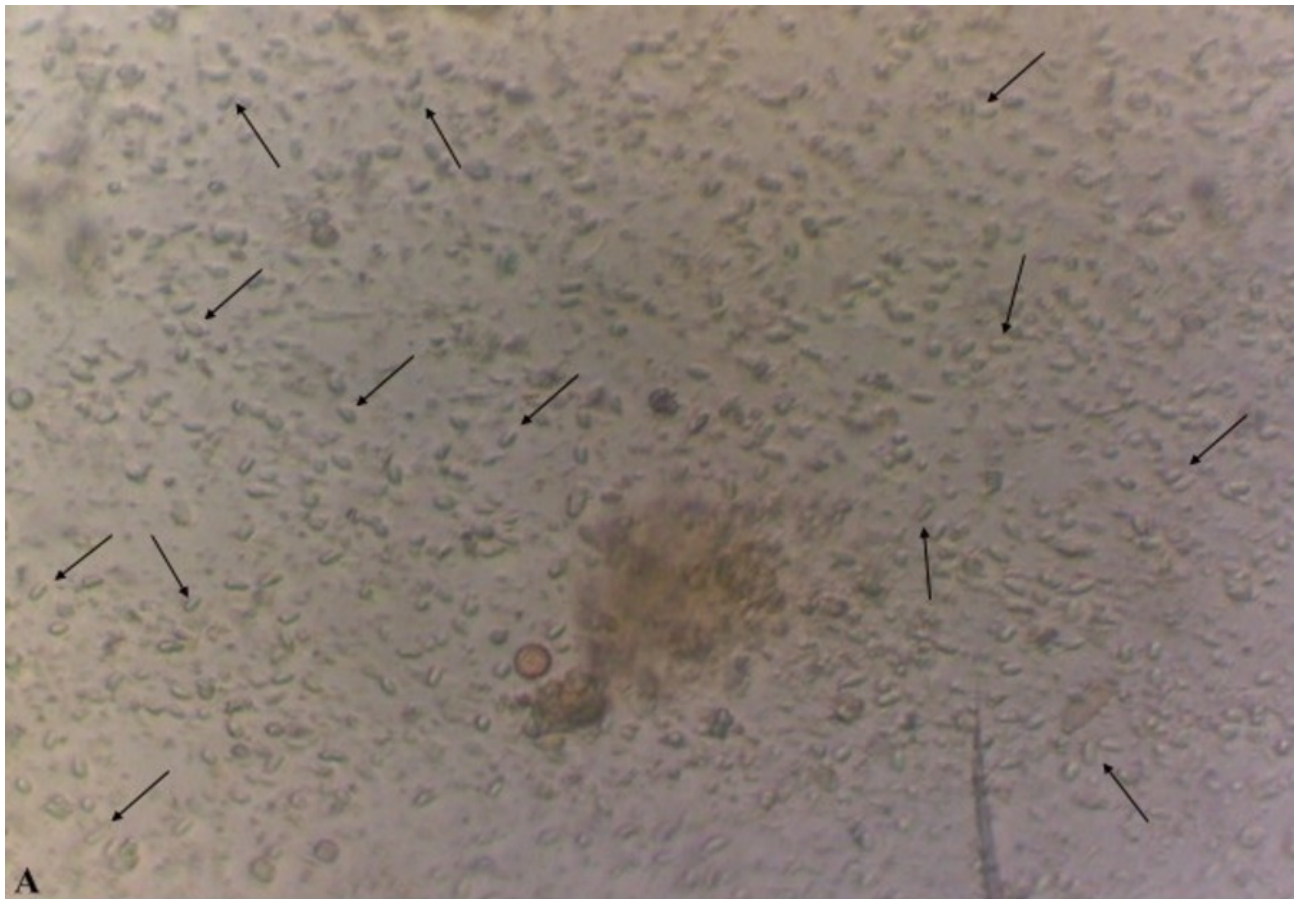



Figure 3

Comparative analysis of the apiaries affected by nosemosis in the districts

(1 – mountainous/steppe; 2 – Katon-Karagay/Urzhar; 3 – Katon-Karagay/Borodulikhinsky; 4 – Katon-Karagay/Shemonaikhinsky; 5 – Urzhar/Borodulikhinsky; 6 – Urzhar/Shemonaikhinsky; 7 – Borodulikhinsky/Shemonaikhinsky)

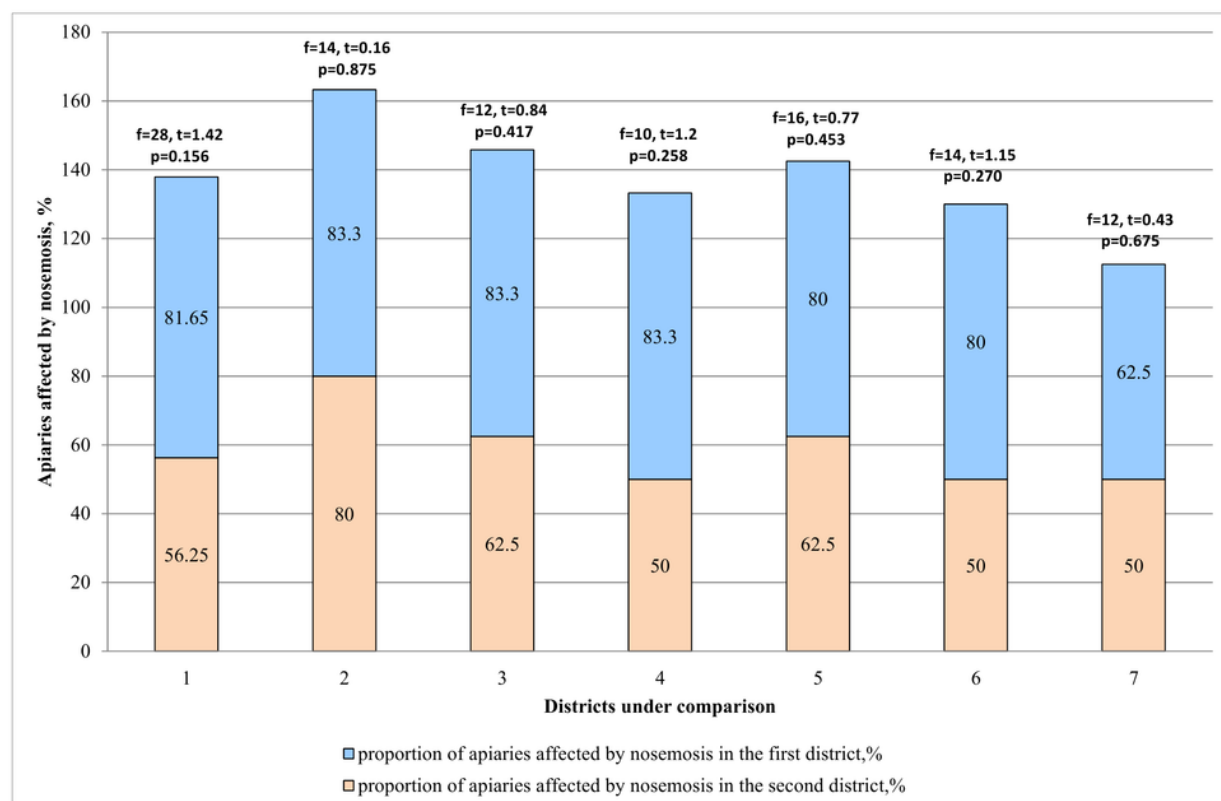



Figure 4

Comparative analysis of the level of infestation of bee families by nosemosis at the apiaries

(1 – mountainous/steppe; 2 – Katon-Karagay/Urzhar; 3 – Katon-Karagay/Borodulikhinsky; 4 – Katon-Karagay/Shemonaikhinsky; 5 – Urzhar/Borodulikhinsky; 6 – Urzhar/Shemonaikhinsky; 7 – Borodulikhinsky/Shemonaikhinsky)

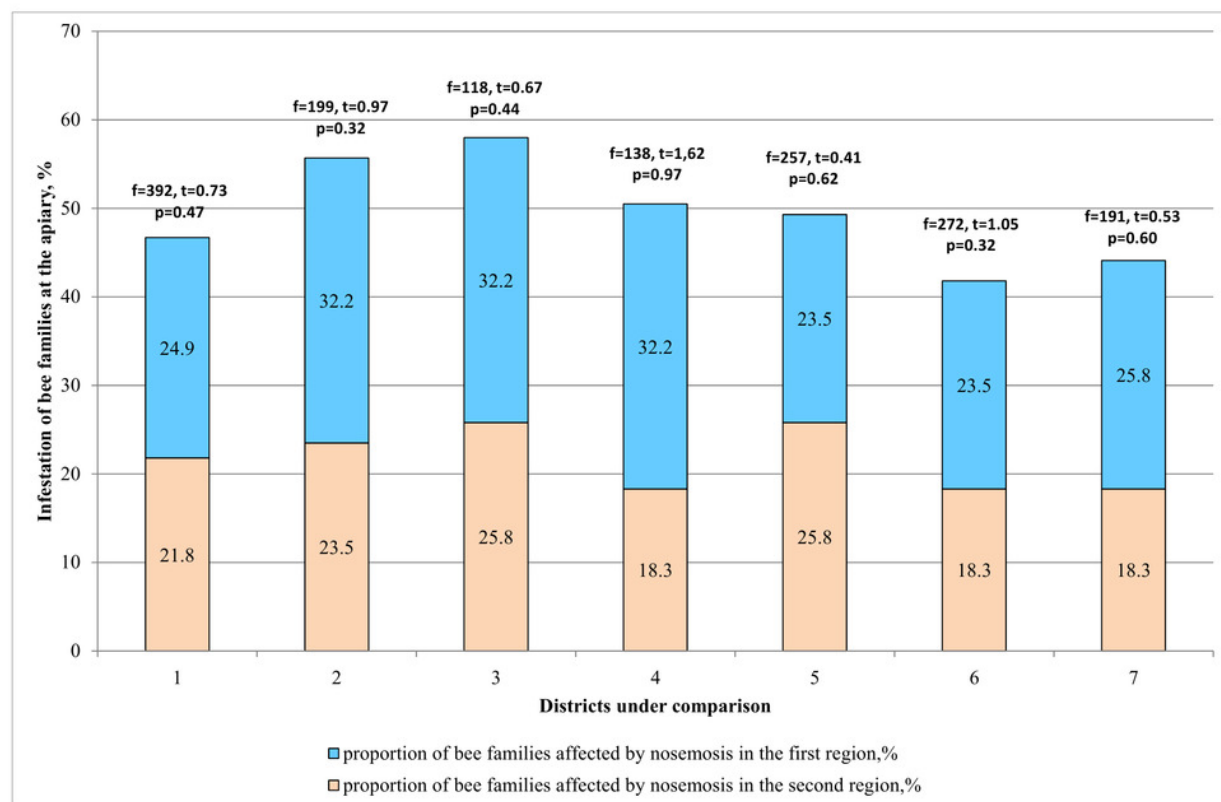
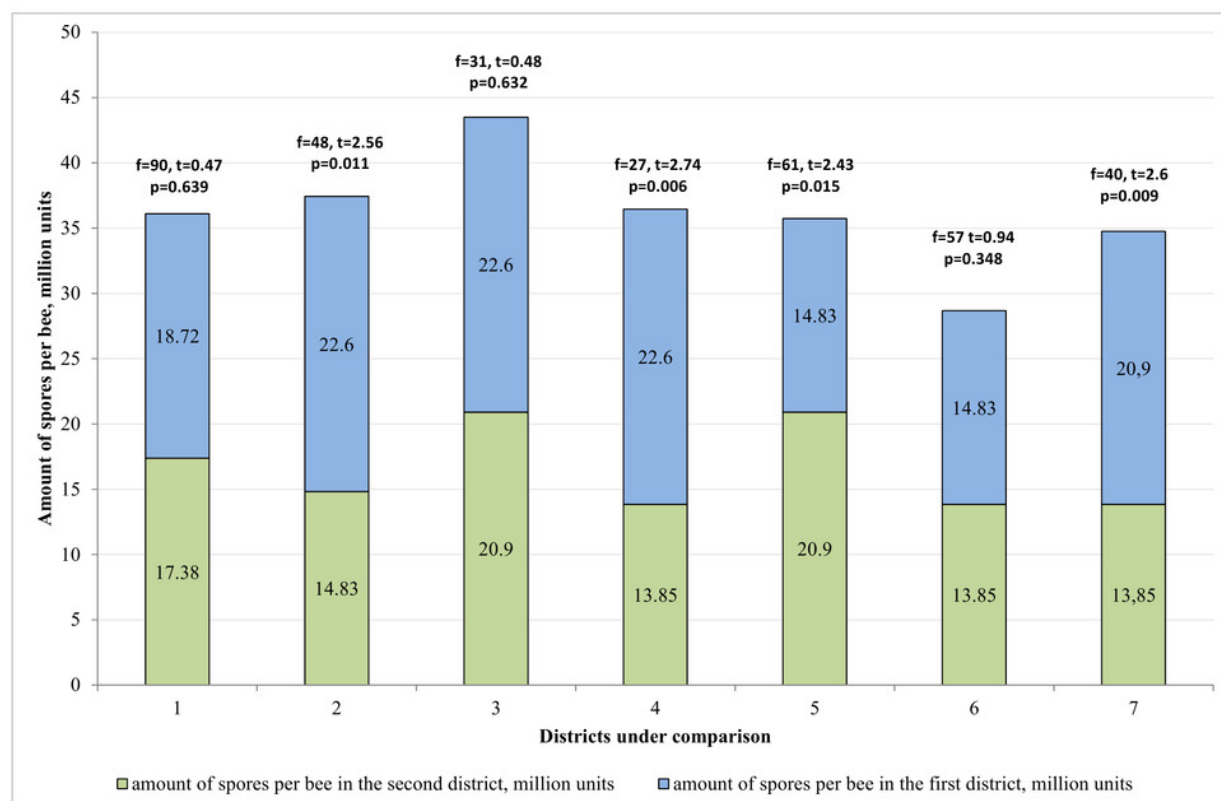



Figure 5

Comparative analysis of the amount of spores per bee

(1 – mountainous/steppe; 2 – Katon-Karagay/Urzhar; 3 – Katon-Karagay/Borodulikhinsky; 4 – Katon-Karagay/Shemonaikhinsky; 5 – Urzhar/Borodulikhinsky; 6 – Urzhar/Shemonaikhinsky; 7 – Borodulikhinsky/Shemonaikhinsky)

Assessment of infestation of the honey bee families by microsporidia of the genus *Nosema spp.* (Zinatullina, 2018)

Level of infestation, points	1 – low	2 – average	3 – high	4 – severe
Amount of spores per bee, million	up to 5	from 5.05 to 25	from 25.05 to 75	over 75

Infestation of apiaries by microsporidia of the genus *Nosema spp.* in different districts of East Kazakhstan Region

Districts of East Kazakhstan Region	Apiaries			Total amount of bee families at the apiaries under study, units
	Total examined, colonies	Infestation by nosemosis, colonies	Prevalence, %	
Katon-Karagay	6	5	83.3	320
Urzhar	10	8	80	1430
Borodulikhinsky	8	5	62.5	892
Shemonaikhinsky	6	3	50	1050
In the Region:	30	21	70.0	3692

Levels of infestation of bee families

Districts of East Kazakhstan Region	Samples			Level of infestation (amount of samples, cases/% from the total amount of samples (% from the amount of positive tests))					Amount of spores per bee, million
	tested, cases	positive		-	1 point	2 points	3 points	4 points	
		cases	%						
Katon-Karagay	31	10	32.2	21/67.5	0/0 (0)	4/12.9 (40)	6/19.4 (60)	0	22.6± 2.76
Urzhar	170	40	23.5	130/76.5	3/1.8 (7.5)	28/16.5 (70.0)	9/5.3 (22.5)	0	14.83± 1.25
Borodulikhinsky	89	23	25.8	66/74.16	2/2.2 (8.7)	9/10.1 (39.1)	12/13.5 (52.2)	0	20.9± 2.17
Shemonaikhinsky	104	19	18.3	85/81.7	1/1 (5.2)	14/13.5 (73.7)	4/3.8 (21.1)	0	13.5± 1.85
Total (mean value*)	394	92	23.4*	302/76.6	6/1.5 (6.5)	55/14.0 (59.8)	31/7.9 (33.7)	0	17.96*