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Seasonally Age Dynamic of Ascospherosis Disease of Bees and Its Progress in East Kazakhstan.

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ABSTRACT

Bee ascospherosis is the infectious mycotic disease causing death of bee and dron brood succeeded by its mummifying. Studying of emergence and flow of ascospherosis in various climatic zones and the areas of East Kazakhstan region demonstrates that the clinical picture of its manifestation on bee garden is in direct dependence on influence of the climatic factors, existence of the forage supply and veterinary–sanitary upkeep of bee colonies. Precise diagnosing of ascospherosis requires to conduct epizootological examination of bee gardens with studying of seasonal and age dynamics and age features of disease.

Keywords: bee, ascospherosis, disease, East Kazakhstan, beekeeping

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INTRODUCTION

Beekeeping - one of the most important and profitable sector of agriculture, where its effective development increase the quality and level of farming agriculture and receive high-quality products.

Since ancient times East Kazakhstan was considered as the favorable place beekeeping. At the end of the 20th century in the East Kazakhstan region there were more than 65% of honey bee colony of all number of bee gardens of Kazakhstan and was produced up to 70% of commercial honey which was generally exported. The Altai honey is praised by quality of honey plant – herbs (polymorphic honey). Therefore, the Altai honey is not so fragrant, but one of the best for healthwise [1].

After Soviet Union collapse the beekeeping in Kazakhstan fell into decay. Kazakhstan lost its position in the world market of honey. The large-scale farms were no longer to grow the tributary, lucerne, sainfoin and other melliferous plants. Moreover the demand for honey was dropped. The honey production had been decreased to 1 thousand tons, and average per capita consumption of this product - to 50 g a year which is 4 times lower than the corresponding average world index.

There was a visible progress in beekeeping of Kazakhstan from 2010. The National union of beekeepers "Ball Ara" was created and a number of programs of revival of beekeeping were developed. The strategic goal of these programs is to turn Kazakhstan into "the beekeeping power" and within the next 10 years to enter of ten leaders of global manufacturers of honey. For this purpose primarily it was planned:

- Creation of the breeding plants and bee gardens;
- Revival of "honey conveyors";
- Expansion of promotion and sales of products of beekeeping in Kazakhstan;
- Honey export improvement.

Beekeeping developing began with the plans of agriculture development of the country. One of the long-term projects is to put the part of the salted soils (about 5 million hectares) under the sweet clover and other melliferous plants and return it for agriculture use.

Since fall of 2011 in the schools and kindergartens of East Kazakhstan regions about 47 thousand children have a honey in their menu every day. Local city government allocate more than one million tenge (3000$) for these purposes. Locally these costs looks small but the effect is enormous.

To increase the production of healthful product, since 2011 the local government started funding of beekeeping in East Kazakhstan. New 12 bee gardens have been created. Serious work on preservation of unique breed of the Central Russian bee which in olden times was in Kazakhstan Altai [2].

BEE DISEASES

Infectious and invasive diseases of bees lead to death and weakening of bee colonies, reduce their quality and efficiency, cause to beekeeping extensive economic damage. Therefore, cure diseases is one of the important tasks of the beekeeper. At present time the progressive veterinary medicine has the sufficient range of drugs, for treatment of diseases of bees. Besides, the beekeepers have to pay attention to prevention of diseases among bees by means of preventive actions both on the bee garden in general, and in any beehive separately.

The most widespread and causing the significant damage to beekeeping of East Kazakhstan region are such diseases as varroatosis (agent of disease – tick Varroadestructor), nosematosis (agent of disease – Nozemaapis microsporidia), ascospherosis (agent of disease – Ascosphaeraapis mushroom) and American foulbrood (agent of disease – Bacilluslarvae bacillus). Above-mentioned diseases are widespread on all bee gardens of East Kazakhstan region and often proceed in the mixed form. The obtained data of bee garden study of East Kazakhstan region showed the stable presence of varroatosis which amounts to 46±1,3% of total infection of honey bee colony. Varroatosis disease by 7 times exceeds the infection of the American foulbrood

– 6±5,8%. The infection of honey bee colony by ascospherosis was 2,4±1,5% and nosematosis was 3,9±1,9% respectively.

The large economic damage for beekeeping causes Ascospherosis disease of bees (calcified brood) – the infectious mycotic disease of bees causing death and male bee brood followed by mummifying.

The first report on this disease was appeared in Czechoslovakia in 1878 [3]. In 1907 in Switzerland registered the outbreak of ascospherosis of bee families [4]. In the subsequent, the disease was revealed in Germany, France, Greece, England, Poland and Italy. Ascospherosis, generally, appeared in spring and at the beginning targeted the small amount of male bee brood. However the disease was not considered serious as it was not widespread and did not cause essential economic damage to beekeeping. The economic damage became notable with spreading and growing the ascospherosis of bees [5, 6, 7, 8,].

If earlier calcified brood was found only in separate colonies, it often stopped by itself, but with time this disease extended everywhere [9].

For the first time the pathogen of ascospherosis was studied on the basis of research of A. Maassen, who in 1918 defined the mushroom causing a disease and called it Pericystisapis [7]. In 1932 A. Betts [10] differentiated the pathogen agent of ascospherosis from the closely related mushroom of Pericystisalver which was described it earlier works [10].

The most detailed works devoted to studying of description and pathogen of ascospherosis belong to P. Claussen [5] and A. Maurizio [11]. C.F. Spiltor and V.S. Olive [12], studied the life cycle of fungus and classified it to the Ascomycetes (marsupials) class, but final systematic position of fungus was not defined[13]. S.F. Spiltor (1955) defined for it a new sort – an askosfer [14]. Later it was proved that spores of A. apis, mixed with the forage and assimilated in bees and germinated in their digestive organ where the mycelium develops and gets through the digestive tract walls and accumulates at the surface of bees body from where it begins to infect the brood [11].

Mycelium A. apis is septate, branchy, multinuclear body of fungus which reaches to 600 microns of length and 3-8 microns thickness; has pores between hyphas in septums therefore internal protoplasm can proceed from the cell to cell [12].

On the nature of gamogenesis A. apis fits into the phycomycetes. It also can be referred to the exoascale fungi due to the multicellular mycelium and emergence of zygote to embryonic sporangium. On the basis of research, the ascospherosis disease agent of bees rank to the class Ascomycetes, subclass Gimnoascomycetidae (Protoscomycetes), Sinascales order, and Pericystaceae family [14].

It is established [15, 16, 19] that spores of A. apis have two thick covers and rather low metabolism that allows to keep viability and patogenicity for a long time.

Viability of spores of A. apis is more than three and less than 35 years [17].

Disease agent spores have stability in the external environment. According to research the fungus remained viable after 15 years of storage and at the temperature of 27 °C it is preserved within one year. The spores of ascospherosis survived for 4 years if they remained in the empty beehives, on the equipment, honeycombs, and also in the perga and honey storing at the bee gardens. Spores are steady against influence of chemicals. So, 1% of hydrogen dioxide kills spores in 30 min., 1% formaldehyde and glutaric dialdehyde - 20 min., 3% solution of lime chloride and hypochlorine - 10 min. [18].

Spores are less steady at the wet way of storage (0,02 g of the crushed died larva + 10 ml of sterile distilled water) and are inactivated at the temperature of 21 - 27 °C after 6 months, and at 40 °C - in 1 month. Low temperatures (-12 °C) preserve pathogen at the wet way of storage for 12 months [19]. Spores of fungus keeps viability in the thermostat at 30 °C, in not heated bee's cell storage at -15 - +20 °C, in the refrigerator at +4 °C within 48 months (observation term) [20].

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BEE DISEASE IN KAZAKHSTAN

Studying the seasonality of infectious and invasive disease episodes in various climatic zones of the Republic of Kazakhstan demonstrates that the epizootic process is changing due to the influence of various climatic factors, phytosanitary condition of forage reserve and beekeeping method.

Each disease characterized by its own epizootic process and particular stages of bees life during the infection. Studying the emergence and the progress of ascospherosis disease in different climatic zones of East Kazakhstan region demonstrates that the aspect of ascospherosis on bee gardens is in direct dependence on influence of climatic factors, existence of a fodder resources and veterinary-sanitary maintenance of bee colony. Bee keeping method plays significant role on bee colony life.

Thus it was observed that by the nature of disease emergence two forms of disease were revealed: latent and mild cases.

The latent form of ascospherosis was observed generally in Urdzhar during the spring and summer period. Most cases of disease self-recovered during the growing of bee colony. At the latent form of disease it had been noted isolated cases of larvae infection and therefore there was not observed the clear clinical picture of the disease.

The mild case of ascospherosis (to 10 – 20 larvae on the bee's cell) at the end of April and beginning of May in Katon-Karagay district (fig. 1), at the end of May in Urdzhar district. With the temperature increase of the environment and improvement of the condition of fodder sources, raising the flight activity of bees and improvement of immune status of bee colony there was a self-recovering of beehives.

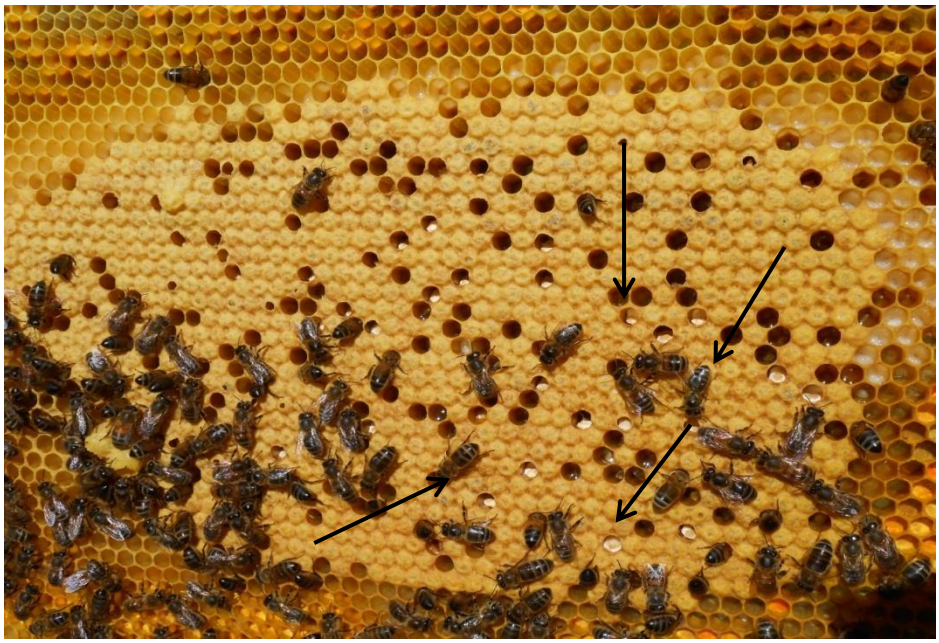

Fig. 1: Mild case of ascospherosis (Katon-Karagay, 2013)

In the period of beekeeping season the relation of clinical symptoms of ascospherosis on the larval bee age was defined (table 1).

Table 1 shows that in April ascospherosis was observed in 1–2-day larvae bee and dron brood 43,4±4,7% and 28,0±2,7%; in July - 8,2±1,6% and 10,2±1,1% respectively. In September 3,8±1,5% and 2,7±1,1% of 1-2-day larvae bee and dron brood were infected by ascospherosis. The peak of infection of larvae bee of 3-4-day age was defined in June and reached 58,4±5,7% and 64,1±5,3% of bee and dron brood respectively. In September 50,7±5,0% and 44,2±4,5% of 5-6 day larvae bee and dron brood had ascospherosis disease which was the highest value in compare with April and June time.

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Table 1: Larval bee brood mortality at ascospherosis *(M±m)*

Brood open cells	Larval bee age, days	April	June	September
True bees	1-2	43,4±4,7	8,2±1,6	3,8±1,5
	3-4	27,7±5,5	58,4±5,7	33,4±4,0
	5-6	16,5 ±2,4	15,1±2,0	50,7±5,0
Dron	1-2	28,0±2,7	10,2±1,1	2,7±1,1
	3-4	41,0±10,6	64,1±5,3	32,2±4,1
	5 - 6	22,0±2,0	19,0±1,2	44,2±4,5

For each infectious and invasive genesis disease of bees the infections of insects specifically occurred at a particular stage of its growing.

The clinical implication and pathomorphologic changes at infection of Ascosphaera apis of bees were studied in vivo at 17 bee gardens of Katon-Karagay, Urdzhar and Borodulikha districts, ten of which unsuccessful on this disease.

In the infected bee cells from death of brood to mummification passed 11-13 days. At the same time if 2-3 day larvae brood was infected, it dead in 4 -5-day age. Clinical symptoms of the disease appeared at 3-4 day. Mummification of the dead brood was happened at 14 - 16 day.

During the observation of pathogenesis progress and clinical symptoms of ascospherosis, it can be associated three stages. Each stage passes a particular time period.

In the first state of the disease where the brood was infected and killed, it was noted that the larvae stretched along the walls of cells, the surface of cuticle paled, segments of larvae was gradually smoothed out, where their consistence then condensed, and the surface of cells remained opened.

During the beginning of the second stage it was noted the mold growth inside the cells and on the surface of larvae. The second stage was the stage of mycelium active growth. In cases when the queen bee put the eggs in honeycomb which was infected by fungus, the process of infection and fungal mycelium formation on the surface of the larvae was occurred in parallel with mycelium growth in the brood.

The mummification of the brood was the third stage of the disease. It was followed by the calcification of larvae, transformation into white-grayish dense lumps (fig. 2).

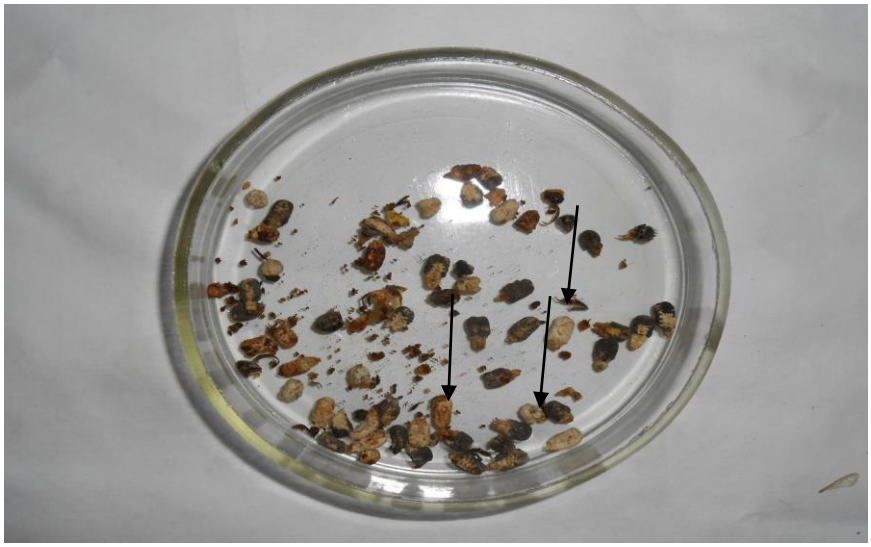

Fig. 2: Third stage of ascospherosis: calcification of larvae

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Table 2: Bees clinical aspects duration stages during the beekeeping period, days (M±m)

Stage	Brood age, days					
	Bees brood			dron brood		
	1-2	3-4	5-6	1-2	3-4	5-6
1	0,9±0,1	1,8±0,4	2,2±0,4	0,6±0,1	1,3±0,2	1,8±0,1
2	3,9±0,9	4,9±0,9	5,9±1,3	2,9±0,9	5,0±0,9	4,0±1,3
3	7,0±1,5	8,0±1,5	13,0±1,5	11,0±1,5	13,0±1,5	10,0±2,5
1-3	14,0±2,9	18,0±2,9	22,0±2,0	17,0±2,0	20,0±2,0	25,0±3,0

According to the table 2, the first stage for 1-2-day dron brood was lasted to 0,6±0,1, and for 3-4-day - 1,3±0,2 days; for bee dron of the same age – 1,8±0,4 days. Duration of the first stage disease of 5–6-day bee and dron brood reached up to 2,2±0,4 and 1,8±0,1 days, respectively. Duration of the second stage of disease for 1–2-day dron brood was 2,9±0,9 days; for bee dron was 3,9±0,9 days. The third stage of disease was preceded longer than previous. Thus, for 1-2 day bee dron the disease was following for 7,0±1,5 days, for 3-4 day bee dron - 8,0±1,5 days, for 5-6 day bee dron - 13,0±1,5 days respectively. The third stage of dron brood was proceeded: for 1-2 day larvae - 11,0±1,5, for 3-4 day larvae - 13,0±1,5, and for 5-6 day larvae - 10,0±2,5 days respectively.

If for 1–2-day dron and bee brood from the beginning to mummification of the disease was passed respectively 17,0±2,0 and 14,0±2,9 days, then for 5–6-day dron and bee brood - 25,0±3,0 and 22,0±2,0 days respectively.

Also, the fungus growth was observed on the sealed cells where its lids were slightly convex. Subsequently, on their surface observed the increase of the white threads separately settling down from each other which with time merged and formed a continuous grayish mold on lids.

CONCLUSION

- By the epizootological monitoring of bee gardens of East Kazakhstan it was established that ascospherosis (disease agent – Ascosphaera apis fungus) – one of the most widespread diseases which cause significant damage to beekeeping.
- It was determined that in spring time the throughout the 1-2 day age bee and dron brood season in spring time by ascospherosis disease were infected 1-2 days bee and dron brood of 43.4±4,7% and 28,0±2,7%, respectively. The highest infection of 3-4-day larvae was detected in June and reached 58,4±5,7% and 64,1±5,3%. In September 50,7±5,0% and 44,2±4,5% of 5–6-day larvae of bee and dron brood had the maximal affect.
- As the result of observation of the pathogenesis pathway and the manifestation of clinical symptoms of ascospherosis, it was allocated three stages of disease. The clinical symptoms were shown by larvae stretched along the walls of cells, the surface of cuticle paled, the mold growth inside the cells smoothing of their segments, and calcification of larvae, and their transformation into white-grayish dense lumps.
- Laboratory diagnostics of pathological samples diagnosed the ascospherosis and identified pure growths of ascospherosis. The ascospherosis disease agent of bees was shown by fungus growth in the form of white fluffy colonies.

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