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CIRCULATION FEATURES OF *Parafasciolopsis fasciolaemorpha* (Ejsmont, 1932) ON THE TERRITORY OF THE VYATKA PRIKAMIE UNDER WEATHER ANOMALIES

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Abstract

The parasite fauna of ungulates is a permanent component of natural biocenoses. Species-specific and most pathogenic moose biohelminth *Parafasciolopsis fasciolaemorpha* Ejsmont, 1932 in the forest zone has epizootic significance, forms stable natural foci. The study of this trematode remains insufficient throughout the entire range of the species. There are few scientific publications on the influence of abiotic factors on the helminth life cycle. The presented work is based on monitoring data of parafasciolopsosis in the Kirov Region. There was statistically confirmed for the first time that moose infection depends on the amount of summer precipitation, and to a lesser extent depends on temperature. The purpose of our work is to establish the features of the incidence of parafasciolopsosis in moose with significant deviations in the temperature and humidity regime of summer seasons. The studies were carried out in the floodplain Cheptsa River (a large tributary of the Vyatka River) within the southern taiga zone of the east of the Russian Plain in 2009-2021. Liver samples of 189 moose were processed by helminthological autopsy. A quantitative account of sexually mature specimens *P. fasciolaemorpha* was carried out with subsequent extrapolation of data. The prevalence of invasion and intensity of invasion in moose were calculated. The infestation of the intermediate host, the freshwater snail *Planorbarius corneus* (L., 1758), was determined by the hepatopancreas compression method with counting the number of trematode larvae. To assess the meteorological conditions of the summer seasons, we used the average air temperature (°C) and the average amount of precipitation (mm) for June-August from open source data of Internet resources for the city of Kirov. Weather anomalies of summer seasons (average air temperature, average amount of precipitation) are expressed as a percentage of the average values for the reference period 1961-1990. With an increase in the abundance of the local population of moose (from 3.1 to 16.7 individuals/1000 ha of forest land), a consistent increase in the incidence of parafasciolopsosis is recorded (from 33.3 % to 76.5 %). A high positive non-linear relationship was established between the abundance of the local moose group and the prevalence of invasion ($r = 0.76$, $R^2 = 0.86$, $n = 9$, $p \le 0.05$). During the study period, there were summer seasons with extreme temperature and humidity regimes: three abnormally rainy, four dry, four cool and five hot years. In dry years, the concentration of the invasiveness in floodplain water bodies increases significantly. An inverse non-linear dependence between the infestation of moose with the trematode *P. fasciolaemorpha* and anomalies in the amount of precipitation during the summer was revealed. The precipitation deficiency provokes an increase prevalence of invasion in the definitive host ($r = -0.60$, $R^2 = 0.89$, $n = 8$, $p \le 0.05$). The temperature factor has a weak effect on the *Parafasciolopsis* infection of the moose: the correlation with the prevalence of invasion is medium positive ($r = 0.31$, $R^2 = 0.75$, $n = 8$, $p \le 0.05$), with the intensity of invasion is medium negative ($r = -0.46$, $R^2 = 0.24$, $n = 8$, $p \le 0.05$). A decrease in the average intensity of invasion was found together with a general high incidence of this trematodosis in moose due to the development of concomitant immunity. To normalize the situation in the parafasciolopsosis focus, it is advisable to selectively shoot weakened male moose during the rutting season. In floodplain lands, it is required to increase the shooting of calves, as the most infected age group, serving as a source of environmental pollution with parasite eggs. In dry seasons, a single application of anthelmintic preparations into licks is necessary.

Keywords: moose, parafasciolopsosis, prevalence of invasion, intensity of invasion, meteorological anomalies, Cheptsa River, Kirov region

In recent decades, weather and climatic conditions are characterized by record anomalies of meteorological parameters during growing seasons (droughts, excessive precipitation, etc.), which causes deviations in the development of plant and animal diseases, imbalance in the dynamic "parasite-host" system, and an increase in the frequency and intensity of parasitic diseases [1, 2].

Among representatives of the deer *Cervidae*, the elk *Alces alces* L., 1758 [3, 4] which has a species-specific parasite in its helminth fauna *Parafasciolopsis fasciolaemorpha* Ejsmont, 1932, is especially susceptible to trematode diseases. The dixenic development cycle of the trematode includes an intermediate host, the aquatic mollusk *Planorbarius corneus* (L., 1758).

The greatest pathogenicity of the disease is manifested in Central and Eastern Europe [5], but throughout the entire range of the species, *P. fasciolaemorpha* is relatively poorly studied. Research on this trematodosis is being conducted in Belarus [6, 7], Poland [8-10] and Latvia [11, 12]. It is known that elk are infected with *Parafasciolopsis* on the territory of Russia in some central regions of the European part (Moscow, Voronezh and other regions) and in the east of the Russian Plain (Kirov region) [13-15]. These works mainly provide data on the extensiveness of invasion (EI), less often on the intensity of invasion (II) and the abundance index (AI). Isolated cases of significant infestation of moose in Central Europe, leading to the death of the animals, have been described, and the histological features of moose livers affected by *Parafasciolopsis* have been described [5, 12, 16].

Modern molecular genetic methods in the study of helminthiases in moose based on the study of fecal samples provide assessment of *P. fasciolaemorpha* prevalence during the growing season and identification of the infection degree in the definitive host depending on sex (the extent and intensity of invasion was higher in males) [10, 17].

Analysis of factors influencing the spread of parafasciolopsosis is mainly limited to assessing changes in the density of moose groups and the abundance of the intermediate host, the composition and quality of natural biocenoses [18]. An increase in the infestation of the main and intermediate host, as well as the fecundity of maritas during the growing season due to seasonal changes in abiotic factors is discussed [10, 19]. Low temperatures in the winter months have a suppressive effect on the fertility of trematodes, reducing it to minimum values [19).

The influence of weather conditions on the circulation of *P. fasciolaemorpha* in natural ecosystems was first characterized by A.S. Rykovsky [20] who established a high probability of an outbreak of parafasciolopsosis in dry seasons due to the movement of moose to water bodies and the accumulation of mollusks in shallow water. Using the example of the central regions of the European part of Russia, there is an increase in the incidence of parafasciolopsosis in moose in dry years by 10-20% [13, 21]. However, in these works there are no statically significant correlation data on the dependence of the infestation of the definitive host on meteorological factors. In the floodplain of the river Cheptsa (Vyatka River basin) a stable focus of parafasciolopsosis is recorded where *P. fasciolaemorpha* represents the core of the elk helminthocenosis, dominating in frequency of occurrence and abundance [4, 18]. Regular observations in the river basin. We have been conducting Vyatka since 2009, but the information received earlier was fragmentary and was not systematic in terms of spatiotemporal parameters.

In the presented work, based on monitoring data on the epizootic situation regarding parafasciolopsosis caused by *P. fasciolaemorpha*, in the Kirov region, for the first time, the high dependence of elk infestation (intensity and extensiveness

of invasion) on the amount of precipitation in the warm season and, to a lesser extent, on temperature factor.

The purpose of our work is to reveal the characteristics of the incidence of parafasciolopsosis in moose with significant deviations in the temperature and humidity regime of the summer seasons.

Materials and methods. The research was carried out in 2009-2021 in the central part of the Kirov Proince on the territory of the scientific and experimental hunting farm of the Professor Zhitkova All-Russian Research Institute of Hunting and Fur Farming (EHF VNIIOZ) with an area of more than 66 thousand hectares, where floodplain lands accounted for 6.5%, forest lands for 65%. The model site was located in the lower reaches of the river Cheptsa (the largest left tributary of the Vyatka River) and covered a wide (up to 4 km) floodplain of the river with a system of oxbow lakes, shallow permanent and temporary reservoirs. The density of the local moose population within the territory under consideration was determined by the method of winter route census of individuals per 1000 hectares of forest land (individual/1000 hectares).

The helminthological dissection method [22] was used to process 189 liver samples from moose killed in the autumn-winter period. A quantitative census of *P. fasciolaemorpha* was carried out, followed by extrapolation of the data. We calculated the extent of invasion (EI, %), the proportion of infected moose in the sample, the intensity of invasion (II, min-max, specimen/individual) is the arithmetic mean number of trematodes in infested individuals, indicating the range of variation in the sample.

The infestation of the mollusks *Planorbarius corneus* was determined by the hepatopancreas compression method with counting the number of trematode larvae [23]. The abundance of mollusks is presented as the arithmetic mean (*M*) with a confidence interval (standard deviation, ±SD).

To assess the meteorological conditions of the summer seasons, an average air temperature (°C) and average amount of precipitation (mm) for June-August, we used data from open access Internet resources [24] for the city of Kirov (60 km west of EHF VNIIOZ). Anomalies of meteorological indicators were expressed as a percentage of the average values for the reference period 1961-1990 (climate norm).

Correlation and regression analysis of the obtained data was carried out using statistical software packages Microsoft Excel and Statistica 12 (StatSoft, Inc., USA). The sample mean and standard deviation of the mean (±SD) for the number of years of observation (*n*) were calculated. The Pearson correlation index (*r*) was used. To assess the quality of linear and polynomial regression models, the coefficient of determination (R^2) was used. The reliability of the obtained data was assessed at the level of statistical significance $p \leq 0.05$.

Results. Modern climatic trends with an increased frequency of extreme weather anomalies are making adjustments to the circulation of trematodes in natural biocenoses. The possibility of a surge in parafasciolopsosis during dry seasons has been considered previously [13, 20] and is confirmed by our studies [3, 25]. This work focuses on the circulation features of the trematode *P. fasciolaemorpha* not only depending on the amount of precipitation, but also on the temperature factor during the alternation of extremely hot and abnormally cold summer seasons.

During the period 2009-2021, there were three abnormally rainy and four dry summer seasons (Fig. 1), as well as four cool (2009, 2015, 2017, 2019) and five hot years (2010, 2011, 2013, 2016, 2021). Four growing seasons were characterized by extreme temperature and humidity regimes in different combinations: a very hot, dry summer of 2010, a cold, very rainy summer of 2017, a cold summer

without precipitation deficit in 2019, and a hot, precipitation-deficient summer in 2021. Dry summer seasons recurred every 1-2 years, while the norm for the Kirov region was once every 3-4 years [26].

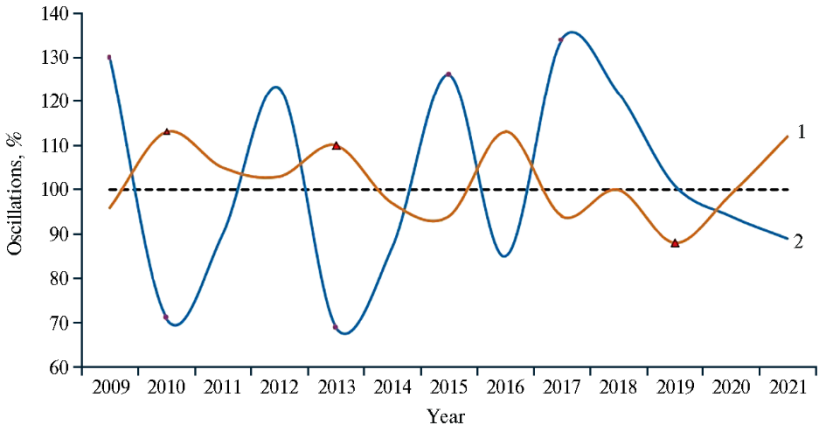

FIg. 1. Anomalies of average air temperature (1) and amount of precipitation (2) for June-August in different years in the central part of the Kirov region (Kirov). The dotted line indicates the norm of the indicator (100%).

An increase in the frequency of weather anomalies with significant changes in temperature and humidity conditions [26] has a significant impact on the quantitative and qualitative indicators of biota development [27], in particular on the behavioral reactions of elk during hot periods [28]. Since 2011, with the relative stabilization of the moose population density in the territory of the EHF VNIIOZ, it has become possible to analyze the cause-and-effect relationships between meteorological parameters and fluctuations in the parafasciolopsis infestation of the definitive host in a short time interval.

In 1996-2000, in the territory of the EHF VNIIOZ, the situation with parafasciolopsosis was favorable with a low density of elk of 3.1±1.1 individuals/1000 hectares of forest land ($n = 5$). The average EI of *P. fasciolaemorpha* was 16% with an average EI of 1038 (134-2087) per individual.

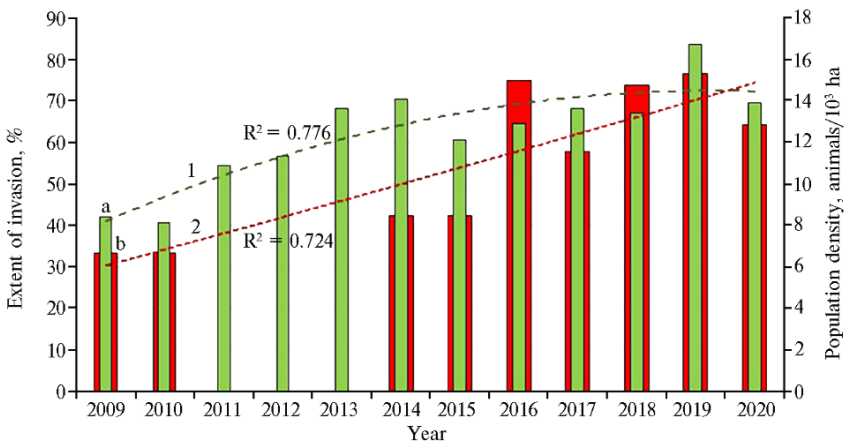

Fig. 2. Absolute values (a) **and polynomial function** (1) **of the local population density of the elk *Alces alces* L., 1758, as well as absolute values** (b) **and polynomial function** (2) **of the intensity of invasion of *Parafasciolopsis fasciolaemorpha* Ejsmont, 1932 over years (NOOH Zhitkov All-Russian Research Institute of Hunting and Fur Farming, Kirov Province).**

After the depression of the 1990s, the number of moose in the territory under consideration steadily increased (by 2014 the increase was 286%), and

subsequently stabilized with small multidirectional deviations under the influence of a number of biotic and abiotic factors [3]. The density of the local moose population has increased 5-fold since the end of the 20th century, reaching 16.7 individuals/1000 hectares in 2019. According to a number of researchers [13, 21], different natural zones with certain landscape, hydrological and climatic features are characterized by different values of the optimal density of elk, the excess of which provokes an increase in the incidence of parasitic diseases. In the conditions of the southern taiga of the European Russia, with an increase in the density of the moose group to more than 9-10 individuals/1000 ha, the risk of an outbreak of parafasciolopsosis significantly increases [13]. This is confirmed by the results of our studies [3, 15]. From 2011 to 2020, in the territory of EHF VNIIOZ, a density of elk grouping formed above the critical one, the 13.3±1.6 (10.1-16.7) individuals/1000 ha (n = 10) (Fig. 2).

With an increase in the abundance of the definitive host, there was a consistent increase in the incidence of parafasciolopsosis in animals. A high positive relationship was established between moose population density and the extent of parafasciolops infestation, r = 0.76 (n = 9, p ≤ 0.05) (Fig. 3).

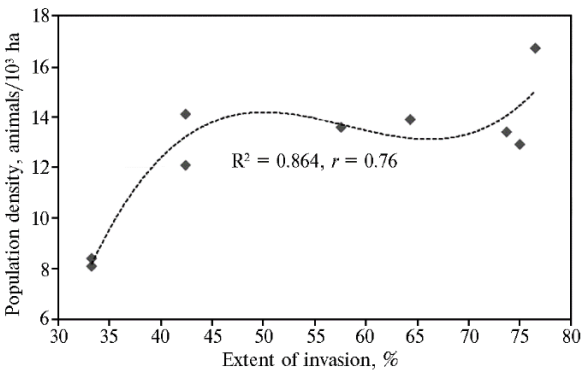

Fig. 3. Correlation field of the abundance of the local population of moose *Alces alces* L., 1758 and the extent of invasion of *Parafasciolopsis fasciolaemorpha* Ejsmont, 1932 (2009-2021, NOOO Zhitkov All-Russian Research Institute of Hunting and Fur Farming, Kirov Province).

The average EI of *P. fasciolaemorpha* for 2009-2020 was 55.39±17.9%, gradually increasing from 33.3% (2009-2010) to 76.5% (2019). The average II for this period is 4305±2694 (17-48984) per individual.

A similar trend is observed in Poland and Latvia, where an increase in the infestation of moose with *Parafasciolopsis* was noted [4, 29]. In Latvia, the infection rate of moose with *P. fasciolaemorpha* ranges from 24 to 42% [12, 29]. In Poland, the incidence reaches 70-100%, and a case of very high intensity of invasion was recorded (more than 10 thousand specimens/individual), which led to irreversible histological damage to the liver and death of the elk [5].

The circulation of trematodes in natural ecosystems is influenced by a complex of abiotic factors (temperature, humidity, lighting, etc.), the synergistic effect of which is ambiguous and in different proportions can stimulate or suppress the development of helminths [30, 31]. In this work, we analyzed the influence of temperature and humidity anomalies in the summer seasons on the circulation of parafasciolopsosis in the southern taiga forests of the Middle Volga region.

On the territory of the Vyatka Prikamye region, during the dry summer seasons of 2010, 2013, 2014, 2016, precipitation fell 15-31% less than the climatic norm, and in the rainy seasons of 2015, 2017, 2018 - 22-40% more. For example, in 2010, there was an abnormally hot, dry summer. In 2013, there was a dry summer with short periods of heat. In the rainy year of 2015, there was a summer flood on all rivers of the Kirov region, and on the river. In Chepetsa, the water rise in August reached 1.4 m. In the cold and rainy summer of 2017, the amount of precipitation exceeded the norm by 35-40%.

The temperature factor influences the behavior of the definitive host of

the marite *P. fasciolaemorpha*. Moose are sensitive to heat stress; on hot days they take refuge under the canopy of trees, and during the cool twilight hours they increase their activity and movement. With further climate warming, changes in the fertility and survival rate of moose, as well as modifications in their behavioral reactions in response to unfavorable weather conditions are possible [28]. Our research has shown that during periods of drought and elevated temperatures, moose more often visit large bodies of water to quench their thirst, cool off, escape from midges and in search of wetland plant food, including the poisonous plant *Menyanthes trifoliata* L. (1753), which promotes circulation bile (moose do not have a gallbladder), pain relief from bites, wound healing. It is in such large bodies of water that the intermediate host of *P. fasciolaemorpha*, the *Planorbarius corneus*, lives. The shallowing of lakes contributes to the accumulation of mollusks in shallow water in the zone of macrophyte thickets. Therefore, in dry years, the concentration of invasive pathogens in the coastal strip of large oxbow lakes increases significantly.

The influence of abnormal seasons in terms of precipitation affected the infection rate of elk after 1-2 years [4]. Thus, the peak intensity of *P. fasciolaemorpha* invasion, the 8610 (19-48984) per individual was recorded in the hunting season of 2014-2015, after the dry years of 2010 and 2013. After the abnormally rainy and cool summer of 2015, during the 2016-2017 hunting season, a minimum intensity of invasion was observed, the 915 (17-3490) per individual, with a decrease in the overall infestation of elk to 57.6%. The cold summer and rainy autumn of 2019 affected the quantitative indicators of parafasciolops infection of elk during the 2019-2020 hunting season. Compared to the previous season, there was a decrease in the occurrence of infected moose to 64.3% with an invasion intensity of up to 3.5 thousand individuals/individual.

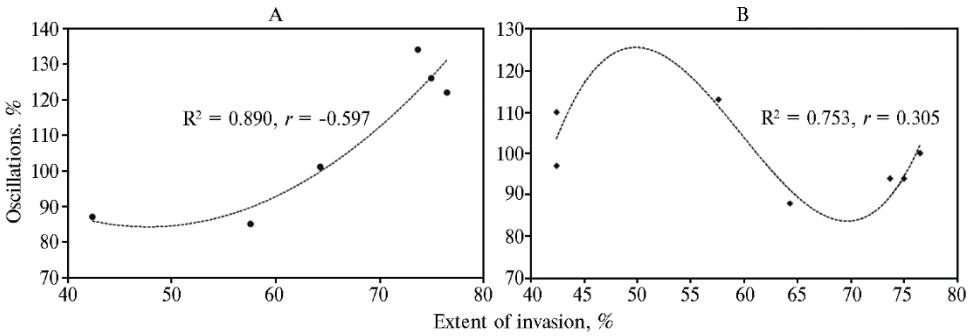

Fig. 4. Correlation fields of the extent of parafasciolopsis infestation of elk *Alces alces* L., 1758 with meteorological anomalies of the summer seasons: A — precipitation, B — average air temperature (2009-2021, NOOO Zhitkov All-Russian Research Institute of Hunting and Fur Farming, Kirov Province).

Based on the results of a conjugate analysis of fluctuations in elk infestation with Parafasciolopsis under the influence of significant deviations from the norm in summer precipitation, an inverse nonlinear relationship was revealed. Deficiency of precipitation provoked an increase in the extent of invasion ($r = 0.60$, $n = 8$, $p \leq 0.05$) (Fig. 4, A). The infection of elk with *Parafasciolopsis* showed an average nonlinear dependence on the temperature factor (see Fig. 4, B). The correlation between temperature anomalies in the summer season and the extent of invasion was averagely positive ($r = 0.31$, $n = 8$, $p \leq 0.05$), with the intensity of invasion - average negative ($r = –0.45$, $n = 8$, $p \leq 0.05$). The weak degree of correlation with the temperature factor is probably explained by the ecological feature of the intermediate host, which inhabits large standing or low-flowing water bodies, where the temperature is not subject to significant fluctuations and the

development of parthenitis occurs in relatively stable interannual conditions. This is consistent with the opinion of UK researchers [32] that temperature within the optimal range does not have a significant effect on the rate of cercariae development in mollusc hosts. However, American scientists [33] have identified changes in the parasite-host system with climate warming: the larval stages of the parasite develop faster in mollusks at elevated temperatures, but there are certain limitations: at temperatures above 30 °C in mid-latitudes, the development of parthenite parasites in mollusks slows down [32].

The fertility of marites *P. fasciolaemorph*a also naturally changes throughout the year depending on the ambient temperature: it is higher in the warm months compared to the winter period. The number of eggs released by the parasite increases during the growing season [10, 19].

Among the many environmental factors that cause changes in parasitic infestations, climatic variables have the greatest influence. Trematodes that have larval stages, living freely in the environment or parasitizing invertebrates (*Arthropoda* and *Mollusca*), are more susceptible to the influence of climatic factors than those helminths in whose life cycle there are no such phases. Abrupt climate changes can impact helminth (fluke) populations and even lead to outbreaks of parasitic zoonoses [1, 34].

Transmission of parasites occurs under conditions of many fluctuating environmental factors. The stage of searching for a host is especially vulnerable when trematode cercariae are exposed to direct external influences of the aquatic environment. According to experimental data (30), cercariae of the littoral trematode Maritrema novaezealandensis died faster at higher temperatures, increased salinity, and when exposed to ultraviolet radiation. Cercariae of *P. fasciolaemorpha* encyst within a few hours after leaving the mollusk and transform into adolescaria, which are more resistant to unfavorable abiotic factors.

The stable circulation of parafasciolopsosis infestation in the territory we are considering was probably caused by the increased population density of the definitive host, which causes the accumulation of invasive material in water bodies, and a combination of abnormal weather conditions - the frequent recurrence of dry seasons: over 12 years of research, 6 years were noted with a deficit of precipitation, of which 4 - abnormally dry. The infection of shellfish by *P. corneus* with parthenites (redia, cercariae) during the study period remained high (EI up to 71%). The average population density of the intermediate host in oxbow lakes was 4.6 ± 3.3 indibiduals/m^2. In years with a deficit of precipitation, the concentration of horny coil in the coastal zone of water areas increased to 15 indibiduals/m^2, and in rainy seasons it decreased to 3 indibiduals/m^2.

The species-specific elk parasite *P. fasciolaemorpha* in the southern taiga forests of the Middle Volga region is a permanent component of the biocenosis, which serves as one of the mechanisms of its ecological balance. Impaired balance can cause an outbreak of parafasciolopsosis. In the context of current climate trends with increasing weather anomalies, it is necessary to take measures to prevent a possible outbreak of this zoonosis. In farms with excessively high densities of elk in limited areas, selective shooting of weakened males during the rutting period, as well as animals with morphological abnormalities and developmental delays, is required. When developing moose quotas, it is advisable to shoot mainly in floodplain lands and mainly young of the year as they are the most infected and serve as a source of environmental pollution with parasite eggs [19, 25, 35].

The organization of anthelmintic activities is especially necessary during dry seasons. According to the recommendations of Belarusian scientists [36], the veterinary drug Triclamizole at a dose of 75 mg/kg can be used once with the laying out of salt licks, the effectiveness of which in moose parafasciolopsosis is

95-100%. To constantly attract moose, it is advisable to locate salt licks near small watercourses and at more than 1.5 km distance from deep oxbow lakes suitable for intermediate host habitat.

Thus, the ability of adult moose to neutralize the impact of *Parafasciolopsis fasciolaemorpha* Ejsmont, 1932 parasites through the development of concomitant immunity, which is maintained through constant contact with the invasive principle, has been established; its interruption even for a short time leads to loss of immune status. This explains the decrease in the intensity of invasion against the background of the overall high extent of invasion by parafasciolopsis. On the territory of the Kirov region, a statistically confirmed high direct dependence of the extensiveness of parafasciolopsis infestation of moose on population density and a high inverse dependence on the amount of precipitation that fell during the summer were revealed. The temperature has a weak effect on the infection of elk with *Parafasciolopsis*.

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