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Analysis of spatial and temporal dynamics of epizootic process of blackleg in Ukraine

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The goal of our research was to identify the model and study the features of blackleg epizootic process of cattle in Ukraine. The analysis of official statistical data of state veterinary reports on the incidence of blackleg infection in the territory of Ukraine over the period 1971–2007 were performed. The method of epizootological analysis was used. Ukraine is the territory of permanent existing of epizootic process of blackleg. Its activity has a temporary irregularity with a tendency to decrease, which is explained by the reduction in livestock numbers, starting from the beginning of the 90s, an increase in the use of the blackleg vaccine in the late 1970s. The growth of the epizootic situation in the early 70s and by the mid-80s was due to intensive land reclamation work, which was accompanied by the removal of the spores of the pathogen blackleg on the soil surface. On the other hand, the introduction of veterinary and sanitary plants for the disposal of destructive raw materials in the late 70s reduced the incidence of blackleg. The most intensive epizootic situation was observed in Lviv and Dnipro (epizootic index 0.64), in Volyn (0.68), in Rivne and Kharkiv – 0.8 and 0.88 respectively. The activity of the stationary foci of blackleg is highest during the first four years and amounts to 80.5% of all repeated outbreaks of the disease. Manifestation of the epizootic process of blackleg in Ukraine has a pronounced seasonality, especially in pasture season, which indicates the soil character of this infection. The epizootic process of blackleg of cattle in Ukraine is characterized by constancy, temporal irregularity, pronounced seasonality and sporadic manifestation, slow extinction of its intensity.

Keywords: Blackleg; Soil infections; Epizootic process; Epizootic situation; Cattle

Introduction

Blackleg (BL), also known as black quarter, is a generally fatal form of myonecrosis usually observed in young cattle (Useh et al., 2006). BL is caused by *Clostridium chauvoei* and according to its epizootic characteristics, etiopathogenesis and clinical manifestation occupies a special niche in the nosological structure of clostridioses (Harwood et al., 2007; Groseth et al., 2011). Clinically diagnosed BL causes significant losses to smallholder households and big dairy farms, and requires animal health authorities to consider serious interventions to prevent further losses from re-emergence of the disease in the known endemically affected areas (Nampanya et al., 2019).

The major virulence factors of *C. chauvoei* have been discovered in recent years but the pathogenesis of blackleg in cattle is yet not fully elucidated (Idrees et al., 2018). Yet, P. S. Pires et al. (2017) invite to speculate on the hypothesis that macrophages are responsible for carrying C. chauvoei spores and even its vegetative cells from the rumen or intestines to muscles in the early stages of the disease.

Clostridium chauvoei is a widespread pathogen that affects ruminants, human and many others animal species. The study of full genome sequences of 20 strains of *Clostridium chauvoei*, isolated from four continents over a period of 64 years (1951–2015), reviled that the major virulence genes including the highly toxic *C. chauvoei* toxin A, the sialidase and the two hyaluronidases are fully conserved as the metabolic and structural genes of *C. chauvoei* (Rychener et al., 2017). The authors consider that the data indicate that the pathogen is a strictly ruminant-associated and it has reached a dead end in its evolution.

To control the disease a method to determine the prevalence of *C. chauvoei* spores on pasture would be useful. However, diagnostic methods effective for clinical cases (culture and biochemical identification) was not applicable in estimating the risk of blackleg on particular pastures from manure or soil samples (Bagge et al., 2009). Detection of *C. chauvoei* by PCR has taken the researchers attention in the last years. They contemplate that PCR would be faster and independent of contaminating flora. Sasaki Y. et al. (2000) proposed single-step PCR system they found to be useful for direct detection of *C. chauvoei* in culture and in clinical materials from animals affected with blackleg. Most of the others authors confirm the PCR efficacy in both cultures and tissues of affected animals (Sasaki et al., 2001; Uzal et al., 2003; Lange et al., 2010; Garofolo et al., 2011), whereas the proper detection of causative Clostridias in the soil, manure and other nonanimal substances are remaining problematic.

In retrospective study of soil-borne diseases of cattle Hang'ombe et al. (2000) found 103 cases of blackleg that were reported between 1985 and 1997. Soil samples from five areas of the country were examined for the presence of genus Clostridium. The authors used direct immunofluorescent assay to examine the soil for Clostridium presence. In this regard, we strongly believe that the information on area-specific epizootic situation and the features of the epizootic process of blackleg distribution may help to elaborate an efficient program to control the disease in cattle and man. Hence, the aim of our work was to study the features of the epizootic process and identify patterns of development of the epizootic situation of the BL in cattle in Ukraine.

Methods

We used the official data of the state reporting agencies, regional departments of veterinary medicine in Ukraine and own results. The manifestation of the blackleg epizootic process in Ukraine was evaluated by indicators of intensity, extensity and duration of the disease manifestation during 1971–2007 in the farms of 25 administrative regions. We also used the state and regional veterinary reports.

The intensity of the epizootic process manifestation was determined by the indicators of morbidity, incidence, death and mortality rate and locality. The morbidity rate was expressed as the ratio of the number of sick animals to the number of susceptible animals in the affected region, incidence rate as the ratio of the number of outbreaks (incidents) of the disease in the period under investigation to the number of susceptible animals in the region, the death rate as the ratio of the number of animals that died from blackleg to the number of susceptible animals in the region. The number of outbreaks (incidents) were defined as a total number for the period under the study. The number of susceptible animals – the average annual number of livestock in each area at the age from six months to four years, as soon as the cattle at this age is the most susceptible to the pathogen.

Mortality was calculated as the ratio of the number of animals that died to the number of sick animals expressed as a percentage. The locality rate was expressed as the ratio of the average statistic number of sick animals to the number of affected farms. This indicator was determined by dividing the number of sick animals on the number of affected farms in each region and country. The extensiveness of blackleg epizootic process manifestation was characterized by an affection and prevalence rates. Affection rate was calculated as the ratio of the number of registered affected points in the region over the period under investigation to the total number of the farms where cattle were kept. The prevalence rate is the ratio of the number of affected farms registered during the year to the total number of farms with cattle. To do this, the affection rate index of a region was divided on the number of years during which the observation period was conducted. Both indicators were expressed in a percentage.

The duration of blackleg epizootic process manifestation was determined by stationary rate, seasonality and seasonal and epizootic index (IE). Stationary rate as an indicator of the duration of an epizootic process in space reflects the recurrence of outbreaks of infection on the same affected farm. Seasonality indicates changes in morbidity rate during certain seasons of the year. The seasonal index shows in how many times the number of sick animals in a season with the highest morbidity rate is greater compared to the remaining seasons and is determined by dividing the number of sick animals in the highest season on the number of animals that got sick during all other seasons of the year. The epizootic index was determined as the ratio of the number of years during which the infection was recorded in the area, to the number of years during which the observations were performed.

Results and Discussion

Data from retrospective studies indicate that since the first description by S. Zertsalov (1891) of an outbreak of blackleg in Ukraine, the country is permanently affected by this infection. The analysis of veterinary data shows that the most complete statistical information on blackleg in Ukraine has existed since 1971. Fig. 1 shows the tension of the epizootic process in Ukraine in 1971–2007 for the frequency of detection of affected farms (infectious outbreaks) and clinically ill animals.

Fig. 1 indicates the existence of long-lasting epizootic process of blackleg in the country. The activity of the process has a temporal unevenness to both the detection of affected farms and to diagnosing clinical disease in animals. At the same time, the trends in the detection of both sick animals and outbreaks of infection tend to sustainable decrease. A number of reasons can explain this.

Firstly, the sharp decline in the number of livestock in Ukraine since the beginning of the 1990s. For instance, by the end of 1971 the number of cattle was 21.4 million, in 1991 – 24.6, and at the end of 2002 – 9.4 million.

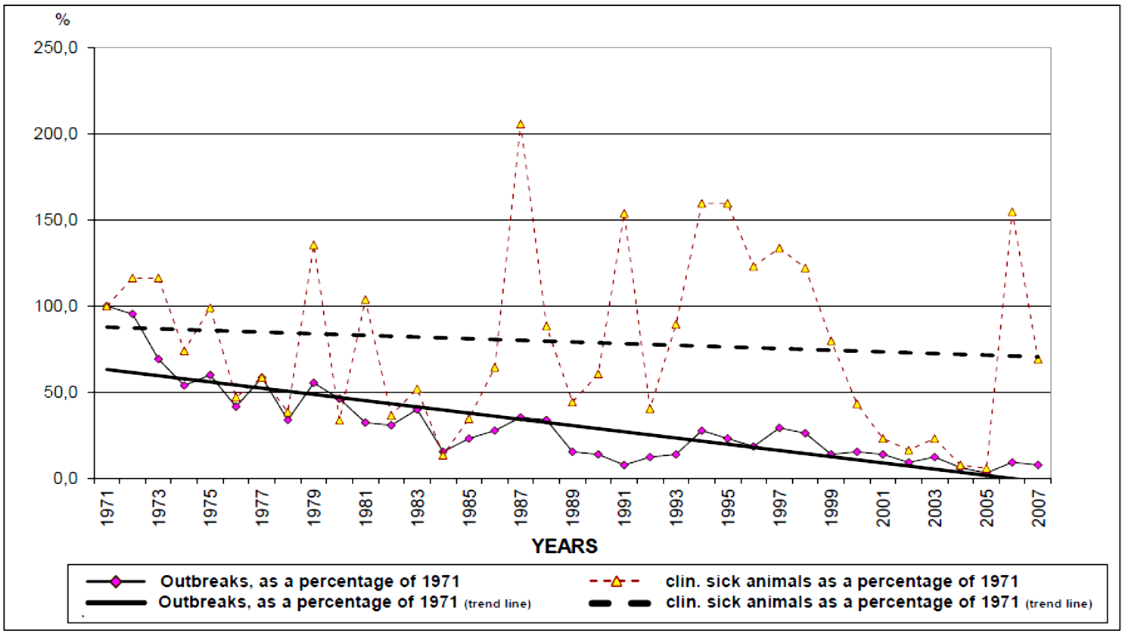

Figure 1. Dynamics of the epizootic process of blackleg in Ukraine (1971–2007).

Secondly, there was a significant increase in the use of a specific blackleg vaccine in affected areas, that is, the implementation of specific preventive measures. For example, the number of vaccinations of cattle in the Volyn, Kyiv and Lviv regions from 49.6 thousand in 1971 increased to 212.7 thousand in 1985. However, in the future, the active immunization of cattle dropped sharply and in the early 2000s was practically stopped, which, accordingly, reflected the clinical manifestation of infection with the high-peak incidence in 2005–2007 (Figure 1).

It is also worth considering the fact that the intensive melioration played an important role in supporting and, in some years, increasing tension in the epizootic process in the early 1970s to the middle of 1980s years. The melioration resulted in a violation of the integrity of the ground surface of natural pastures. This was accompanied by the removal of blackleg spore to the surface with the subsequent infection of susceptible animals. For example, in Volyn oblast, the largest amount of meliorated land was observed in 1980 (62.6 thousand hectares); in subsequent years the meliorated areas started to decrease and within the 90-s almost fully descended. A similar dynamic of meliorated areas was in all regions of Ukraine. Other scientists (Boiko, 2009) also reported the similar close correlative connection between excavation work and increased blackleg incidence.

The others scientists (Bagge et al., 2010) were studying the samples of biogas plants waste and found that even after digestion, the numbers of clostridia decreased, but none of the pathogenic bacteria did and as soon as these residues are used as fertilizers, there is a need to study the prevalence of pathogenic bacteria in such material and its potential spread on the field. According to Sathish & Swaminathan (2008) clostridia microorganisms may be a potential contaminant of carcasses and widespread in soil of abattoir environments.

The increase in the clinical manifestation of infection in the early 90's (Figure 1) may also be due to reduced vaccination level and poor quality of the vaccine (Boiko, 2002). The aluminum hydroxide concentrated vaccine that was developed by F. I. Kagan and A. I. Kolesova in the late 1950s and was used to vaccinate cattle against the blackleg in Ukraine. The vaccine consisted of formaldehyde-inactivated mixture of exotoxine, spores and vegetative forms of *Cl. Chauvoei* (Kagan & Kolesova, 1963). The vaccine provided immunity for up to 6 months (Tretiakov, 1973). The low immunogenicity of this vaccine may apparently be one of the reasons for poor performance of the blackleg eradication program in Ukraine. For instance, in eight regions of Ukraine during 1986–2006, among the vaccinated cattle population, outbreaks of clinical manifestation of the infection were observed 17 times (72 animals out of 3502 vaccinated were affected) (Busol et al., 2010).

Studying the potency of blackleg vaccine P. Nicholson et al. (2019) concluded that haemolysin-neutralizing antibodies are a valuable measurement for protective immunity against blackleg and have the potential to be a suitable replacement of the guinea pig challenge potency test.

Uzal (2012) agreed that scientific evidence on the efficacy of vaccination against *C. chauvoei* to prevent diseases and lethality in cattle is scant and a greater participation of practitioners in clinical research and greater access to informational tools such as systematic reviews must be part of the objectives of veterinary medicine.

Blackleg remains one of the major bacterial diseases that cause noticeable economic losses to cattle farms in many parts of the world. For example, Ayele et al. (2016) found that an overall cumulative incidence and mortality rate of blackleg in local cattle heards was 17.9% (95% CI 16.5–19.4) and 3.6% (95% CI 2.9–4.4%) respectively. Cumulative incidence and mortality rate attributed to the disease in crossbreds cattle was 19% (95% CI 16.9–21.6) and 3.9% (95% CI 2.9–5.3%) respectively. A financial cost in infected herds was estimated to be USD 9.8 (95% CI 6.7–14.4) per head for local zebu and USD 16 (95% CI 10–24.4) per head for crossbred cattle.

Results of the study submitted Wolf et al. (2017) suggested that blackleg cases are clustered within certain geographic areas, which might be due to soil type and water permeability. The authors believed that it should motivate farmers to vaccinate cattle against *Clostridium chauvoei* in known areas with high risk.

The study of the development of the epizootic situation of blackleg in Ukraine by the method of establishing a linear trend for the indicators of infection outbreaks and its clinical manifestation indicates a tendency towards its slow reduction. However, the new wave of the epizootic situation aggravation aroused in 2002–2006. Firstly, it showed the existence on the territory of the country of factors that support the circulation of the pathogen in the cattle population. Secondly – the absence of specific prevention measures (cessation of cattle vaccination against the blackleg) and the weakening of general measures (the reduction of the use of carcass utilization plants). The suggestion is confirmed by the data on the tension of the blackleg epizootic situation in the regions of Ukraine and the Autonomous Republic of Crimea (Table 1).

Table 1. Regional intensity of blackleg epizootic process (1971–2007).

	Region	Cases	Affected animals	Number of death	Epizootic Index
1	Republic of Crimea	17	80	66	0.44
2	Vinnitsa	22	128	122	0.44
3	Volyn	74	249	183	0.68
4	Dnipropetrovsk	30	110	109	0.64
5	Donetsk	35	206	115	0.6
6	Zhytomyr	45	44	44	0.48
7	Transcarpathian	6	84	26	0.16
8	Zaporozhye	5	12	12	0.2
9	Ivano-Frankivsk	24	75	72	0.44
10	Kievskaya	8	45	22	0.12
11	Kirovograd	22	118	118	0.4
12	Lugansk	16	152	147	0.4
13	Lviv	46	62	62	0.64
14	Nikolaev	12	75	73	0.4
15	Odessa	23	168	103	0.6
16	Poltava	13	75	47	0.32
17	Rivne	88	92	91	0.8
18	Sumy	38	136	128	0.32

19	Ternopil	44	230	181	0.6
20	Kharkiv	41	221	210	0.88
21	Kherson	8	20	16	0.2
22	Khmelnitsky	22	59	42	0.44
23	Cherkassy	7	78	40	0.28
24	Chernivtsi	42	166	148	0.6
25	Chernihiv	48	378	277	0.72
	Total	736	3063	2454	1.00

It is evident that blackleg was diagnosed on the territory of all regions and the Crimea (Table 1). In IE – the ratio of the number of years during which the infection was recorded on the territory to the number of years during which the surveillance was conducted, the mentioned administrative territories can be divided into 3 groups. The first group includes areas with IE 0.12–0.37 (Kiev, Transcarpathian, Zaporizhia and Kherson, Cherkassy, Sumy and Poltava), to the second with IE numbers 0.38–0.62 (Kirovograd, Lugansk and Mykolaiv, the Crimea Republic, Vinnytsia, Ivano-Frankivsk, Khmelnytsky and Zhytomyr). The most intense epizootic situation of this indicator was observed in the third group. Namely, in Donetsk, Ternopil, Odesa, Chernivtsi, Lviv, Dnipropetrovsk, Volyn that IE reached 0.68, Rivne and Kharkiv– 0.8 and 0.88, respectively. We also characterized the intensity and extensiveness of manifestation of the epizootic process of blackleg in the context of the regions of the country for the period 1971–1995 (Table 2).

Table 2. Intensity and extensiveness of epizootic process of blackleg (1971–1995).

	Region	*Incidence	*Morbidity	*Death	Mortality, %	Affected farms, %	Distribution, %
1	Republic Krim	4.34	12.49	10.86	86.96	1.52	0.061
2	Vinnitska	3.27	16.68	15.82	94.85	1.26	0.050
3	Volyn'	4.14	20.72	17.87	86.25	1.16	0.046
4	Dnipropetrovsk	3.71	14.52	14.06	96.81	1.57	0.063
5	Donetsk	5.35	8.21	7.45	90.70	2.15	0.086
6	Zhytomyr	5.71	10.48	10.09	96.36	1.76	0.070
7	Zakarpatska	3.27	13.08	13.08	100.00	0.83	0.033
8	Zaporizka	0.98	3.33	3.33	100.00	0.52	0.021
9	Ivano-Frankivsk	2.77	18.61	11.88	63.83	0.88	0.035
10	Kyivska	1.00	7.68	3.51	45.65	0.48	0.019
11	Kirovograd	4.22	26.97	25.57	94.78	1.69	0.068
12	Lugansk	2.90	23.18	16.18	69.79	1.27	0.051
13	Lviv	9.02	12.23	12.23	100.00	2.31	0.092
14	Mykolaivska	2.41	18.77	16.36	87.18	1.04	0.042
15	Odesa	3.98	28.74	18.62	64.78	1.83	0.073
16	Poltavska	2.18	12.60	7.89	62.67	0.67	0.027
17	Rivnenska	9.17	16.68	16.12	96.67	3.20	0.128
18	Sumska	8.06	26.48	26.25	99.13	2.22	0.089
19	Ternopilska	5.81	37.07	29.56	79.74	2.46	0.099
20	Kharkivska	5.60	25.51	23.37	91.61	1.77	0.071
21	Kherson	1.23	3.20	2.22	69.23	0.68	0.027
22	Khmelnitsky	4.12	9.17	7.11	77.55	1.52	0.061
23	Cherkaska	1.59	17.66	9.06	51.28	0.84	0.034
24	Chernivetska	13.59	35.38	29.40	83.10	6.76	0.271
25	Chernigivska	5.64	45.59	26.95	59.11	2.29	0.092
	Total	4.35	18.41	14.64	79.52	1.63	0.065

*per 1000,000 population

The average incidence rate for blackleg in Ukraine during the study period was 4.35 per 100,000 population (Table 3). It was the highest in Chernivtsi (13.59), Rivne, Lviv, Sumy, Ternopil, Zhytomyr, Chernihiv, Kharkiv and Donetsk regions. All of the named regions differ significantly in their geographical location and climatic conditions. Therefore, the incidence index alone cannot reveal the spatial patterns of manifestation of the blackleg during a comparatively short observation period. The death rate for the blackleg in Ukraine is closely correlated with the incidence rate (r>0.9) and the average death rate is 14.64 per 100,000 population. Mortality is an indicator characterizing the severity of the disease. Its average value for Ukraine is 79.56% and in some regions (Zaporizhia. Transcarpathian and Lviv) it reaches 100%. This may indicate a low level of diagnosis of the disease as soon as when diagnosing made timely the disease can be cured. The affection and distribution of the disease (indicators of the extensiveness of manifestation of the epizootic process) show the ratio of the number of affected farms to the total number of the farms in the region during the observation period (affection) or during the year (distribution) respectively. In Ukraine, the affection rate is 1.63% and the highest rate is in Chernivtsi (6.76%). This may be because this area has the smallest number of farms. We have determined that the average annual frequency of outbreaks of blackleg for every 100 thousand km^2 was 3.35. The highest level was in the Chernivtsi region (13.8), high level (more than 6) was in Lviv (8.26), Ternopil (6.96), Rivne, and Sumy regions. The low average annual rate of outbreaks of emphysematous carbuncle was registered for Transcarpathian (0.16), Kherson (0.79), Zaporizhia (0.73), and Kyiv (1) regions. One of the important indicators characterizing the duration of a manifestation of the epizootic process in separate place is stationarity – an indicator that reflects the recurrence of outbreaks of infection in the same affected farm or epizootic center. A detailed analysis of the collected data allowed us to detect 30 affected farms in 14 regions of Ukraine, where, over certain periods, repeated outbreaks of blackleg were observed (Table 3).

Table 3. Data on repeated outbreaks of blackleg in Ukraine (1971–1995).

Index	Periods (years), the repeated cases of the blackleg were registered									
	1	2	3	4	6	7	9	10	19	27
Incidence	23	6	2	2	1	1	1	2	2	1
%	56.09	14.63	4.88	4.88	2.44	2.44	2.44	4.88	4.88	2.44

Incidence - quantity of the repeated cases of the blackleg in the same farms

Data of Table 4 indicates that repeated outbreaks of the blackleg have been observed 23 times during the first year since the detection of the infection; during the second – 6 times; in subsequent years – 1–2 times. On the two affected farms, repeated outbreaks of infection during the study period were observed in 19 and 27 years. In the latter case, the disease appeared as a result of the introduction of a pathogen of infection with peat from the field, where 27 years ago the animal died from emphysematous carbuncle.
Thus, the activity of stationary epizootic centers of the blackleg in Ukraine is the highest during the first four years and makes up 80.5% of all the cases; during the next 23 years – only 12.2% of all repeated outbreaks of the disease.
The above data suggests that the specificity of the duration of the epizootic process of the blackleg, despite the precautionary measures taken in affected farms (disinfection, utilization of corpses, active immunization of susceptible livestock), is expressed by noticed stationarity. It can be due to the vegetation of the spore of the infectious agent and, thus, the maintenance of a certain level of its population in affected territories. It is also worth mentioning here of the high resistance of the spore of the pathogen to adverse environmental factors. In our case, the repeated outbreaks of the disease happened in the prolonged periods (19–27 years). The possibility of reproduction of the pathogen in the soil is indirectly supported by the facts of the higher incidence in the summer months. The analysis of the seasonal manifestation of infection indicates that the blackleg in Ukraine is registered in all seasons. However, the prevalence of infection for each month of the year has their own particularities (Table 4).

Table 4. Seasonal dynamics of the blackleg (1971–2007).

Seasons	**Months**	**Affected farms**		**Clinical manifestation**	
		Quantity	**% of total number**	**Quantity**	**% of total number**
Winter	December	32	4.4	279	9.1
	January	44	6.0	210	6.9
	February	51	7.0	143	4.7
Spring	March	49	6.6	304	9,9
	April	65	8.8	180	5.9
	May	72	9.8	136	4.4
Summer	June	74	10.0	474	15.5
	July	71	9.6	184	6.0
	August	73	9.9	338	11.1
Autumn	September	96	12.9	536	17.5
	October	65	8.8	167	5.4
	November	46	6.2	112	3.6
Total		736	100	3063	100

The data in Table 4 shows that the increase in the number of outbreaks of infection begins in April and lasts until October, reaching the maximum level (96 outbreaks) in September. During this period, almost identical monthly records (an average of 74 per month) are observed. Vis-a-vis the monthly average number of outbreaks per year is 61. The seasonal index of the outbreaks of blackleg in Ukraine for the period under study is 1.21. The total relative number of outbreaks occurred from April to October was 69.8%. Beginning from October, there is a gradual decrease in the number of outbreaks, which reaches the lowest peak in December (32 outbreaks) and it is kept at that level (an average of 44 cases per month) until March. This suggests that the manifestation of the epizootic process of blackleg in Ukraine in terms of the number of registered affected farms has a marked seasonality with the higher level of outbreaks occurring in the summer season. Usef et al. (2006) also found the relationship between outbreaks of blackleg in cattle and annual rainfall.
Clinical manifestation of the blackleginfection has a more contrasting picture. During one year, there is one prolonged rise in the clinical manifestation of infection – June-September months. As our previous studies showed, during this period, active use of pastures takes place. The conditions in the pastures are more favorable for the introduction of the pathogen into the body of a susceptible animal.
The second reason for cattle vulnerability on the pastures is the presence of highly susceptible animals. The susceptibility may rise due to injuries to the mucous membranes, which occurs during the grazing period (Bomko et al., 2018; Slivinska et al., 2018; 2019). At this time also, the young calves born in winter acquire the factory fattening conditions and lose colostral immunity (Gutyj et al., 2017; 2018; Kovalenko et al., 2020; Grymak et al., 2020; Borshch et al., 2020). Both of these conditions may facilitate the development of the infection.

Conclusion

The epizootic process of blackleg in cattle is characterized by continuity, temporal unevenness of intensity, sporadicity of manifestation, the presence of a general tendency to the slow decrease of its intensity. In every region, there are areas with a high index of blackleg epizooty, which testifies the existence of conditions that contribute to maintaining the epizootic process. The peculiarity of manifestation of the epizootic process of blackleg is the expressed stationarity. The highest rise of the outbreaks of blackleg is observed during the first four years and accounts for 80.5% of all repeated outbreaks. Blackleg is registered all year-round with a distinct seasonal peaks during the summer months.

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