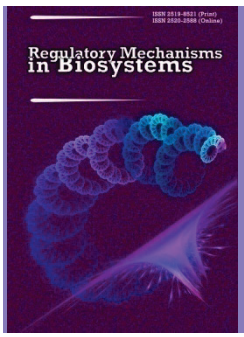

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Retrospective analysis of the epizootic situation of enzootic bovine leukosis in Ukraine in 1994–2019

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In Ukraine, prevention and control measures of bovine leukosis were regulated by relevant legislation, regulations and instructions developed in the period 1960–1992. Currently, the instruction on prevention and rehabilitation of this disease in cattle is used (approved in 2007). According to it, the identification of infected animals is carried out from 6 months of age by serological (first scheme in AGID or another in ELISA) and genomic (PCR) methods. The infected animals are removed from the herd or slaughtered. In general, because of the diagnostic and preventive measures carried out during the analyzed period (26 years) 10 519 farms were rehabilitated from leukosis (2 346 affected farms remained from previous years). At the same time, more 4 million infected cattle were slaughtered. The majority of affected farms (more than 1 000) were registered in the period between 1994 (2 346 farms) and 2003 (1 247 farms). Since 2014, the number of affected farms has remained mostly below 10 and the number of infected cattle has decreased to 2 000 animals per year. At the same time, the number of rehabilitated farms also decreased (from 1 307 farms in 1998 to 4 farms in 2014). The same trend was registered with the dynamics of the number of animals that were removed from the herd due to leukosis. Thus, in 1995 and 1997 their numbers were 321 178 and 558 649 animals, respectively, and in 2014 it was 1 124. The obtained indicators of intensity and extensiveness of the epizootic process show that the incidence rate was maximal during 1998–2000 and amounted to 3.7–4.3%. The maximum indicators of the coefficient of affection were recorded in 1997–2000 and equal 11.8–15.3%. The rate of foci remained on the level of 90–270 throughout the all analyzed period. During 2008–2019, specialists of the Ukrainian veterinary laboratories investigated more than 47 million samples of cattle blood sera for enzootic bovine leukosis by AGID and ELISA. However, despite the significant diagnostic work, the important factor in the decrease of the number of affected farms and infected animals is the decrease in the total number of cattle in Ukraine (almost 22 million animals in 1994 against 3 million in 2019). Graphic trends of these indicators are comparable and agree with the decrease in the number of cattle in our country by analyzed period. After 2014, the number of affected farms ranged 10–17 per year (mostly in private households). However, the full recovery of cattle in Ukraine from bovine leukosis has not taken place, although our country is closer than ever to this.

Keywords: bovine leukemia virus; cattle; epizootic process; serological studies; indicators of intensity and extensiveness.

Introduction

Enzootic bovine leukosis (EBL) is a chronic infectious disease of cattle that is caused by an oncogenic virus of the family Retroviridae. EBL is characterized by a violation of the maturation of the blood cellular elements, malignant growth of hematopoietic and lymphoid tissues, the formation of tumours in various organs, which causes dysfunction of various organs and body systems. Usually, the development of clinical signs in about 5.0% of infected animals begins several years after infection and develops in cattle older than 2–3 years of age. Because of this, bovine leukosis is mostly manifested as a subclinical infection (Berg et al., 2015).

The viral nature of this disease was established in 1969 based on virological studies and detection of viral particles in organs of patients with lymphosarcoma and experimentally infected animals using an electron microscope (Miller et al., 1969). Two years later, the presence of specific antibodies in the blood of infected animals was demonstrated and serological tests for diagnosis of enzootic bovine leukosis in cattle were proposed (Miller & Olson, 1972). Serological tests quickly took a leading place in the system of control and prevention of this disease, showing much higher efficiency than hematological studies (Ferrer et al., 1976; Straub, 1978). Subsequently, the following serological tests were developed to detect specific antibodies: immunofluorescence (IF) (Burny et al., 1978), indirect immunoperoxidase assay (IPA) (Ressang, 1976), complement fixation test (CFT) (Miller & Van der Maaten, 1974), gel-immunodiffusion test (Chander, 1976) and radioimmunoassay (Schmerr et al., 1980). Agar gel immunodiffusion assay (AGID), which is based on the detection of the membrane glycoprotein gp51, in contrast to the viral capsid protein p24, was the most effective (Onuma et al., 1975).

The development of enzyme-linked immunosorbent assay (ELISA) in the early 1990s and its commercialization with the widespread use of AGID, made it possible to increase the efficiency of serological diagnosis of leukosis (Roberts et al., 1989; Reichel et al., 1998). In addition, some modification of ELISA allowed specimens of milk and urine to be investigated (Carli et al., 1993). Both methods, AGID and ELISA, are currently

recommended by the World Organisation for Animal Health (OIE, 2018) for the diagnosis of bovine leukosis as primary and can be used for research at the individual and population levels. The development of molecular genetic methods in the late 1990s enabled studies to be conducted on this disease which are based on the direct detection of DNA in whole blood leukocytes through use of polymerase chain reaction (PCR) (Naif et al., 1990). Currently, the following modifications of PCR were developed that allow detection of the bovine leukemia virus (BLV): nested PCR (Klintevall et al., 1994; Fechner et al., 1996) and real-time PCR (RT-PCR) (Rola-Łuszczak et al., 2013). According to recommendation of OIE, these modifications have been recommended for the diagnosis of bovine leukosis as reference reactions (OIE, 2018). Molecular genetic methods allowed the presence of 10 different genotypes of BLV to be established in different countries (Polat et al., 2017). Genotypes 4, 7 and 8 circulate in Ukraine (Rola-Łuszczak et al., 2013).

Historically, the systemic control for enzootic bovine leukosis and its prevention in the world began in 1960s. By this time, EBL was already a common disease in dairy herds in Northern and Eastern Europe and North America. In 1964, the first recommendations of OIE for this disease appeared. According to them, it was recommended for all countries to create programs for controling the incidences of leukosis, to avoid trade with affected farms and to widely use hematological diagnostics for identification of sick animals (Berg et al., 2015). Subsequently, national eradication programs were adopted in most European countries that are based on the large-scale serological diagnosis by AGID and ELISA (More et al., 2017). In 2017, most countries of the European Union had the official free status concerning enzootic bovine leukosis by the criteria of OIE and the EU Council Directive of 64/432/EEC. According to these documents, more than 99.8% of cattle herds in the country should be free of leukosis. Hungary, Romania, Bulgaria, Greece, Croatia, Malta and some regions of Portugal remain not officially free of this disease (Fig. 1) (More et al., 2017).

In the United States, 46.5% of dairy herds are infected by BLV (LaDronka et al., 2018). By comparison, this indicator in Canada is almost 90.0% (Nekouei et al., 2016). Leukosis is still registered in cattle from Asia (Yang et al., 2016) and North America (Polat et al., 2016). It also continues to be registered in countries neighbouring, Ukraine, including Russia, Belarus, Moldova and Turkey (Zubova et al., 2018; Makarov & Lozovoy, 2020).

Currently, there are no effective vaccines and schemes of treatment for infected animals, so the disease causes significant economic losses on farms, which include the reduction of the animals' productivity and their removal from the herd. All of the above significantly inhibits the development of industrial livestock (OIE, 2018). Although EBL is currently not included in the list of zoonosis due to lack of evidencesof the possibility of human infection caused by the virus, active research in this area is being conducted. According to the data of scientific literature, the potential of this disease as a zoonosis is quite significant (Axel, 2017; Juliarena et al., 2017; Buehring et al., 2019).

The experience of countries that have successfully acquired an officially free status like Italy (Feliziani et al., 2018), Lithuania (Acaite et al., 2007), Finland (Nuotio et al., 2003) and the study of successful implementation aspects of health measures in Croatia (Lojkić et al., 2013) and Japan (Kobayashi et al., 2020) confirms that the most effective methods of rehabilitation are periodic and systemic diagnosis by AGID and ELISA among livestock and removal from the herd or slaughter of infected animals.

In Ukraine, prevention and control measures of bovine leukosis were regulated by relevant legislation, regulations and instructions developed in period 1960–1992. Currently, the instruction on prevention and rehabilitation of this disease in cattle is used (approved in 2007). According to it, the identification of infected animals is carried out from 6 months of age by serological (first scheme in AGID or another in ELISA) and genomic (PCR) methods. The infected animals are removed from the herd or slaughtered (Mandygra, 2000). Evidence of the effectiveness of this strategy is demonstrated by the successful eradication of the disease in many Western European countries (Nuotio et al., 2003; Acaite et al., 2007; Lojkić et al., 2013; Maresca et al., 2015; Feliziani et al., 2018).

However, despite the above-mentioned control and prevention measures, Ukraine has not officially been free of enzootic bovine leukosis for several decades. Therefore, taking into account the epizootic situation regarding bovine leukosis in Ukraine, the authors aimed to analyze the epizootiological aspects of this disease over the past 26 years (there are complete statistical reports on this disease for these years) and critically assess the achievements and problems in this area.

Materials and methods

The authors conducted a retrospective analysis of the epizootic situation regarding enzootic bovine leukosis in Ukraine over 1994–2019. For this purpose, we studied, systematized and analyzed the reports of regional laboratories of State Service of Ukraine on Food Safety and Consumer Protection (reporting form 1-Vet.) and obtained data of the State Scientific and Research Institute of Laboratory Diagnostics and Veterinary and Sanitary Expertise (SSRILDVSE, Kyiv, Ukraine) for the period 26 years.

Epizootological and analytical research methods were used, and indicators of intensity and extensiveness of the epizootic process were analyzed (incidence rate, coefficient of affection, rate of foci).

The incidence rate was defined as the ratio of the quantity of infected by BLV animals to the total number of cattle susceptible to leukosis.

The coefficient of affection was defined as the ratio of the number of farms affected by BLV to the total number of farms.

The rate of foci was defined as the ratio of the quantity of infected cattle by BLV to the number of farms affected by this virus.

Information about the total number of susceptible cattle and farms was obtained from the State Statistics Service of Ukraine.

Results

Overall, during 1994–2019 about 4 million animals sick with enzootic bovine leukosis and 8 thousand farms affected by BLV were found in Ukraine. In general, because of the diagnostic and preventive measures carried out during the analyzed period (26 years) 10 519 farms were rehabilitated from leukosis (2 346 affected farms remained from previous years). At the same time, 4 389 114 infected cattle were slaughtered (Table 1).

The largest number of cattle infected by the bovine leukemia virus was registered in 1994 (474 596 animals), 1997 (404 704), 1998 (470 572), 1999 (505 591) and 2000 years (425 109 animals).

The majority of affected farms (more than 1 000) were registered in the same years: in 1994 – 2 346 farms, in 1995 – 1 801, in 1996 – 2 485, in 1997 – 3 703, in 1998 – 4 469, in 1999 – 4 011, in 2000 – 3 452, in 2001 – 2 454, in 2002 – 1 787, in 2003 – 1 247 farms. Graphic trends of these both indicators are comparable and logically agree (Fig. 1).

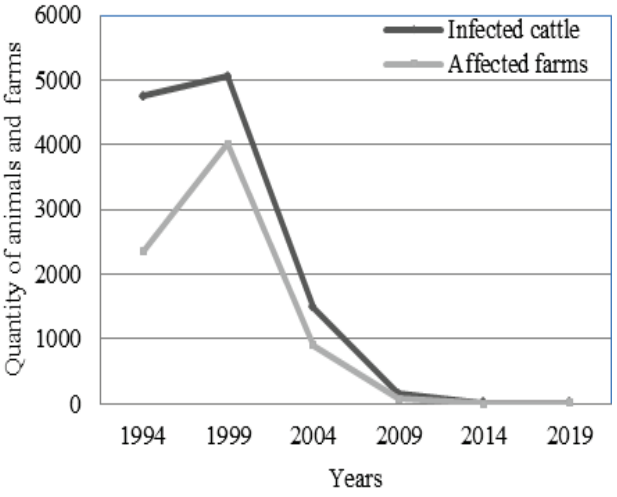

Fig. 1. Dynamics of affected farms and cattle infected by BLV registration by years (for convenience in the trends comparison, the number of livestock per million heads divided by conventional 100 units) according to reports of regional laboratories and SSRILDVSE

Table 1
Summary data regarding rehabilitation measures on farms affected by BLV according to reports of regional laboratories and SSRILDVSE

Years	Data as 01.01 of the current year		Data obtained during the year		Number of rehabilitated farms	Number of slaughtered cattle
	number of affected farms	number of infected cattle	number of affected farms	number of infected cattle		
1994	2 346	474 596	628	206 307	1 173	443 505
1995	1 801	237 398	1 278	346 718	594	321 178
1996	2 485	262 938	1 811	493 111	593	351 345
1997	3 703	404 704	1 798	624 517	1 032	558 649
1998	4 469	470 572	849	578 837	1 307	543 818
1999	4 011	505 591	625	430 689	1 184	511 171
2000	3 452	425 109	227	360 264	1 225	502 096
2001	2 454	283 277	82	234 730	749	237 057
2002	1 787	280 950	40	163 357	580	230 049
2003	1 247	214 258	204	158 163	554	224 125
2004	897	148 296	83	100 756	419	135 878
2005	561	113 174	157	75 654	222	101 942
2006	496	86 886	104	48 477	240	75 134
2007	360	60 229	134	44 001	287	70 487
2008	207	33 743	52	22 390	179	41 666
2009	80	14 467	14	8 629	47	14 979
2010	47	8 117	9	3 790	26	5 828
2011	30	6 079	14	3 036	30	5 325
2012	14	3 790	1	333	13	3 678
2013	2	445	7	1 359	7	1 397
2014	2	407	8	2 061	4	1 124
2015*	6	1 344	9	2 096	6	1 786
2016*	9	1 654	3	910	2	900
2017*	10	1 664	15	1 280	8	1 414
2018*	17	1 530	10	1 295	18	1 238
2019*	9	1 587	25	2 791	20	3 345
Total		8 187		3 915 551	10 519	4 389 114

Note: * marks parameters presented without taking into account the occupied territory of the Autonomous Republic of Crimea, the city of Sevastopol and part of the temporarily occupied territories of Donetsk and Luhansk regions.

As presented on Figure 1, both indicators have been declining rapidly since 1999. This trend continued until 2014, after which the values increased slightly, but the number of affected farms has remained below 10 (only in 2018, 17 were registered) and the quantity of infected cattle has decreased to 2 000 animals per year.

At the same time, the number of rehabilitated farms also decreased (from 1 307 farms in 1998 to 4 farms in 2014). The same trend was registered with the dynamics of the number of animals that were removed from the herd / slaughtered due to leukosis. Thus, in 1995 and 1997 years their numbers were 321 178 and 558 649 animals, respectively, and in 2014 it decreased by 286 times (only 1 124 head).

The number of cattle removed from the herds / slaughtered in 2001 decreased by 2.2 times in comparison to 1999 (237 057 against 511 171 animals). As a result of the diagnostic and preventive measures carried out, in 2006, their number was less than 100 thousand, in 2010 – less than 10 thousand and in 2016 it was only 900 slaughtered animals. However, after that the number of such animals increased again slightly and in 2019 amounted to 3 345 head (Fig. 2).

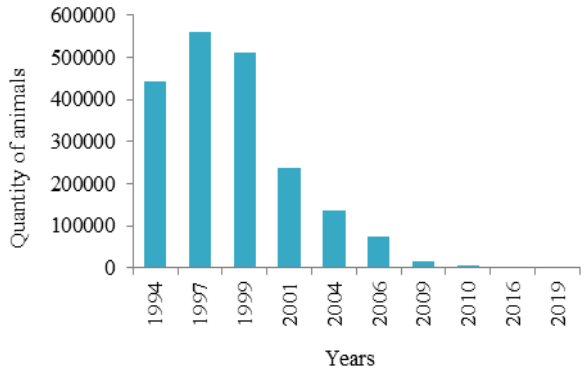

Fig. 2. Dynamics of cattle removed from the herd / slaughtered due the leukosis infection according to reports of regional laboratories and SSRILDVSE

The obtained indicators of intensity and extensiveness of the epizootic process show that the incidence rate was maximal during 1998–2000 and amounted 3.7–4.3%. The maximum indicators of the coefficient of affection were recorded in 1997–2000 and equal 11.8–15.3%. The rate of foci remained on the level of 90–270 throughout the analyzed period.

It should be noted that the rates of incidence and affection began to decline particularly rapidly after 2007, when a new instruction was approved, which paid considerable attention to serological diagnostic methods (AGID, ELISA). Thus, from 2008 to 2019 inclusive, the values of these indicators were less than 1.0%, and after 2012 – above 0.1%.

The values of these coefficients are explained by the fact that at the beginning of 2007 in Ukraine 360 affected farms were registered, in which there were 60 229 head of cattle infected by BLV. The rate of foci was 167 animals per farm. The incidence rate in this year was 1.2% and the coefficient of affection – 1.0%. After the adoption and application of more effective and sensitive diagnostic methods, at the beginning of 2008 there were only 207 affected farms, which is 57.5% less than in the previous year, and 33 743 sick animals (almost twice less than in the previous year). Systematized results of serological investigation conducted after the approval of the current instruction of Ukraine regarding the prevention and rehabilitation of cattle from enzootic bovine leukosis in 2007 are presented in Table 2.

In total, for the period 2008–2019, more than 47 million blood sera samples from cattle were studied for EBL in Ukraine. As presented in Table 2, almost all of these sera samples were studied by the agar gel immunodiffusion assay (AGID). At the same time, 42 times fewer samples were examined by using enzyme-linked immunosorbent assay (ELISA). However, despite such a significant difference in number of investigated samples, only 14 times fewer positive reactions were detected by ELISA (21 003 samples against 294 292). These results are primarily explained by the fact that samples by ELISA were mostly studied that had previous positive reactions by AGID (arbitration or repeated research).

In addition, 77.6% of the total numbers of samples were tested by ELISA in 2019, when enzyme-linked immunosorbent assay test-systems began to be widely used in regional laboratories of the State Service of Ukraine on Food Safety and Consumer Protection.

Table 2
Summarized data regarding serological studies of bovine leukosis according to regional laboratories and SSRILDVSE reports in Ukraine over 2008–2019

Years	Number of investigated sera	Studies in AGID		Studies in ELISA	
		total quantity	number of positive	total quantity	number of positive
2008	5 945 305	5 923 858	69 702	44 863	2 765
2009	4 943 114	4 901 892	39 830	29 528	2 320
2010	4 598 570	4 594 568	35 789	24 249	1 182
2011	4 493 820	4 493 271	30 952	22 124	1 105
2012	4 360 989	4 358 421	23 219	26 714	2 946
2013	4 050 300	4 043 184	15 365	15 229	1 435
2014	3 894 753	3 884 818	18 973	13 609	596
2015*	3 614 176	3 607 529	13 048	9 067	549
2016*	2 895 153	2 888 010	9 818	8 094	211
2017*	2 997 292	2 991 129	10 837	8 139	370
2018*	2 859 776	2 818 742	15 184	46 667	622
2019*	2 720 703	1 864 169	11 577	858 323	6 902
Total	47 373 951	46 369 591	294 294	1 106 606	21 003

Note: * – see Table 1.

In general, there is a positive trend compared to the early 2000s, which indicates the effectiveness of the implemented measures to combat bovine leukosis. However, one of the important factors in this is also that the total number of cattle, and cows in particular, is significantly declining every year. Thus, the number of cattle decreased from almost 22 million to 3 million, while the number of cows also decreased by 4 times (Fig. 3).

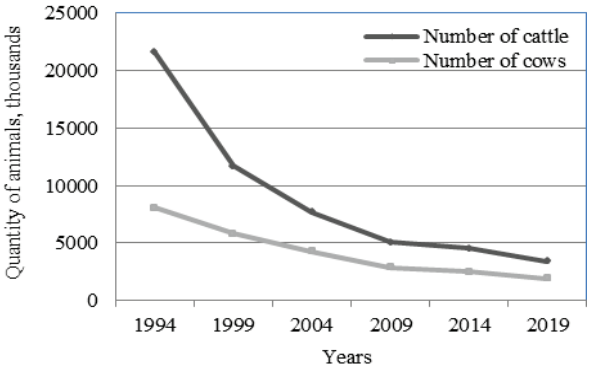

Fig. 3. Dynamics of number of cattle and cows by years according to reports of regional laboratories and SSRILDVSE

Simultaneously with these indicators, the number of serological studies, removed from the herd animals and rehabilitated farms also decreased (Fig. 4).

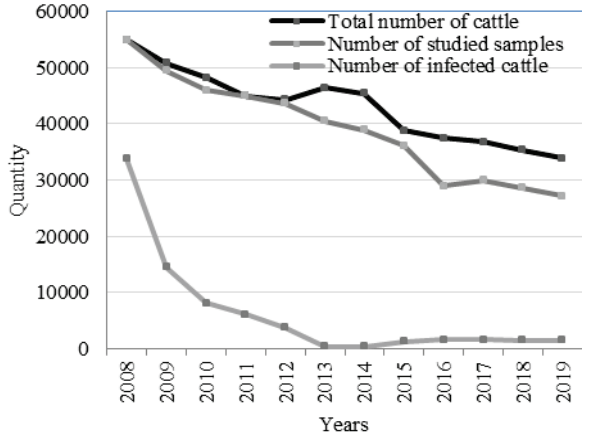

Fig. 4. Dynamics of total number of cattle, studied sera samples and number of infected cattle by years (for convenience in the trends comparison, the quantity of livestock per million head and studied samples divided by a conventional 100 units) according to reports of regional laboratories and SSRILDVSE

All of this certainly indicates the effectiveness of preventive measures and implemented serological methods. However, it should be borne in mind that the total number of cattle also decreased actively in the same years.

Discussion

The first cases of bovine leukosis in Ukraine were registered and described in Kharkiv region (Eastern Ukraine) in 1953 (Stegniy et al., 2013). Later, there were reports of the disease among cows in the western and central regions of our country.

In the second half of the 1980s in Ukraine, according to investigation of Mandygra (2016), there was an extremely difficult epizootic situation with enzootic bovine leukosis. Thus, during a serological study of 5 133 samples from breeding bulls of 126 state breeding stations, 12.7% of infected animals by BLV were detected. In particular, the infection rate of animals from Zaporizhzhya station was 50.0%, Donetsk – 36.5%, Kirovohrad – 16.3%, Volyn – 17.7%, Kherson – 16.5%, Odessa – 16.0%, Ternopil – 15.5%. In the same period, 16.9% of cows that were kept on 139 state breeding stations could not be used for reproduction because of seropositivity to leukosis. The disease began to be registered in the cows from the southern regions of country (41.1% of infected animals from breeding stations in Mykolaiv region) (Dombrovskyi et al., 2003).

The investigation of Dombrovskyi et al. (2003) shows that the epizootic situation of leukosis in Ukraine during 1980–1987 was characterized by large-scale and uneven spreading. It was registered throughout the territory of the country. The incidence of infection (per 100 thousand studied animals) during the analyzed period was relatively stable and amounted to 131.7–247.0 sick cattle. The indicators of intensity and extensiveness of the epizootic process also had high rates: the rate of foci was 0.1, the coefficient of affection – 1.0, the incidence rate – 0.014 (Dombrovskyi et al., 2003).

The epizootic situation began to change after the implementation into veterinary practice of AGID with specific leukosis antigen in 1986. Thus, the rate of infection of cattle in Ukraine was highest in 1986 and amounted to 18.4%. Large-scale diagnostic studies using AGID helped to reduce this indicator to 6.4% in 1994. It increased slightly between 1995 and 1997 but, in 1998, this rate was 6.2%. Thus for 13 years, the rate of infection decreased by almost three times (Mandygra et al., 2016).

As the results of our research have shown, AGID remains the actual and effective method of leukosis diagnosis, despite the implementation into veterinary practice of ELISA and PCR in 2007. During 2008–2019, more than 46 million blood serum samples were tested by this reaction, which is 97.9% of the total number of investigated samples.

At the same time, just over 1 million samples were tested by ELISA (2.3% of total number of investigated specimens). This is due to the fact that ELISA test-systems are more expensive than reagents for AGID, and using them requires special equipment (washer, thermoshaker, reader). Therefore, ELISA was used in most cases only as an additional or arbitration method, when cattle owners expressed a desire to conduct repeated studies. However, taking into account prospects, the State Service of Ukraine on Food Safety and Consumer Protection has begun to actively purchase ELISA test-systems for regional laboratories of Ukraine in 2019 and this trend will continue.

The results of our work indicate that since the 2000s, and especially since 2008, the number of cattle infected by enzootic bovine leukosis and

the number of farms affected by BLV has been actively reduced. The obtained data generally coincide with the results of other authors' retrospective studies (Stegniy et al., 2013).

Particularly promising is the investigation of milk samples pools by ELISA. Thus, the specialists of the Research Institute of Laboratory Diagnostics and Veterinary and Sanitary Expertise (SSRILDVSE, Kyiv, Ukraine) during 2009–2018 conducted an experimental study of 47 072 sera specimens and 688 pools of milk (contained 58 203 individual samples). Samples were collected from cattle in Kyiv region. As a result, 136 positive on EBL serum samples were detected (0.3% of total number of investigated specimens) and 47 milk pools (contained 3 770 individual samples).

If antibodies to the BLV were detected in the milk pool, serum samples from all animals from which the milk was taken were tested. Thus, 55 positive samples of blood sera in 47 positive pools were found, which amounted to 0.1% of the total studied. It was found that even in a pool that includes 96 milk samples, one positive sample was detected.

As for PCR, although it was included in the new instruction, this reaction has not been widely used yet for the diagnosis of the disease in Ukraine. Laboratory staff use this reaction only for investigation in the rare cases of samples from particularly valuable breeding cattle. However, the rapid reduction in the cost of diagnostic kits for PCR gives reason to predict the expansion of this method in the future. This could significantly increase the effectiveness of the studies, because PCR can detect infected calves younger than 6 months of age, despite the potential presence of colostral antibodies in the blood of such animals.

Systematized data regarding the effectiveness of prevention and diagnostics measures throughout the analyzed period indicate that Ukraine was closest to full recovery from enzootic bovine leukosis in 2013–2014, when 2 affected farms and, respectively, 445 and 407 animals infected by BLV were registered. The incidence rate and the coefficient of affection were also 0.01%. However, now the situation regarding this disease is beginning to deteriorate. In 2019, 9 affected farms and 1 587 infected animals were registered.

Conclusions

A retrospective analysis of the epizootic situation regarding enzootic bovine leukosis in Ukraine showed that it was extremely severe in 1994. This year, there were 2 346 farms affected by BLV and 474 596 sick animals, but in the end of 2019 there were only 9 affected farms and 1 587 infected animals, mostly in the private households. The obtained indicators of intensity and extensiveness of the epizootic process have been declining rapidly since 1999. This trend continued until 2014, after which the number of affected farms ranged from 10 to 17 per year. However, the full recovery of the Ukraine from bovine leukosis has not taken place, although our country is closer than ever to this.

The rates of incidence and affection began to decline particularly rapidly after 2007, when a new instruction was approved, which paid considerable attention to serological diagnostic methods (AGID, ELISA). Thus, from 2008 to 2019 inclusive, the values of these indicators were less than 1.0%, and after 2012 – above 0.1%. The incidence rate was maximal during 1998–2000 and amounted to 3.7–4.3%. The maximum indicators of the coefficient of affection were recorded in 1997–2000 and equal 11.8–15.3%. The rate of foci remained on the level of 90–270 throughout the all analyzed period.

In general, according to the reports of regional laboratories of State Service of Ukraine on Food Safety and Consumer Protection and SSRILDVSE (Kyiv, Ukraine) during the analyzed period (1994–2019) 10 519 farms were rehabilitated from leukosis and 4 389 114 infected cattle were slaughtered. The trend of decrease in the number of sick animals and affected farms practically follows the graphic trends of decrease in the total number of cattle in Ukraine.

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