Bovine leukemia virus occurrence in Dagestan

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SUMMARY
Results of diagnostic tests for bovine leukosis carried out in the Dagestan Republic in 2022 (as of October 1, 2022) showing bovine leukemia virus (BLV) occurrence are presented in the paper for the whole Republic and for each municipality separately. In total, 632,454 susceptible animals were serologically tested with immunodiffusion assay; 3,573 reactor animals (0.6% of tested animals) were detected. Proportion of infected animals was as follows: 0.5% – in administrative raions, 1.6% – in urban districts and 1.1% – in distant pasture zones. Percentage of infected cattle varied from 0.01 to 4.9%. No hematological examinations for bovine leukosis were carried out because seropositive animals were timely culled. In the breeding sector, the proportion of bovine leukemia virus carriers was averagely 2.8%. Ninety-five bovine leukosis-affected localities were reported in the region as of 1 January 2022. Eighty-one new BLV-infected localities had been identified and bovine leukosis had been eliminated in 18 localities for 9 months of 2022. Totally, 158 localities were officially declared affected in 2022 (as of October 1, 2022): 36 agricultural holdings (including 5 breeding holdings), 18 small-scale farms and 104 backyard farms. The largest number of bovine leukosis-affected localities was registered in the Kizlyarsky (18), Tarumovsky (17), Babayurtovsky (16), Gunibsky (15), Tlyaratinsky (10) Raions and in the city of Makhachkala (9). One disease-affected locality was reported in each of the Bezhtinsky, Buynaksky, Derbentsky, Kazbekovsky, Kayakentsky, Kizilyurtovsky, Khasavyurtovsky Raions and towns of Khasavyurt and Yuzhno-Sukhokumsk. Two disease-affected localities were reported in each of the Rutulsky, Untsukulsky Raions, three disease-affected localities were reported in each of the Gergebilsky, Laksky, Novolaksky, Tsumadinsky Raions. Four disease-affected localities were reported in the Sergokalinsky Raion, five disease-affected localities were reported in the Charodinsky Raion, six disease-affected localities were reported in each of the Akhvakhsky, Dakhadaevsky, Karabudakhkentsky Raions, seven disease-affected localities were reported in each of the Botlikhsky, Kumtorkalinsky and Shamilsky Raions. Comparative analysis of serological and molecular genetic methods used for bovine leukosis diagnosis demonstrated the advantage of enzyme-linked immunosorbent assay and polymerase chain reaction as compared to immunodiffusion assay used in veterinary practice.

Keywords: bovine leucosis, bovine leukemia virus, occurrence, immunodiffusion assay, enzyme-linked immunosorbent assay, polymerase chain reaction, Republic of Dagestan

Степень распространения вируса лейкоза крупного рогатого скота в Дагестане

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РЕЗЮМЕ
Изложены результаты диагностических исследований на лейкоз в Республике Дагестан в целом и отдельных муниципалитетах за 2022 год (по состоянию на 1 октября), характеризующие распространенность вируса бычьего лейкоза. Всего за анализируемый период подвергнуто серологическому исследованию в реакции иммунодиффузии 632 454 восприимчивых животных, выявлено 3573 положительно реагирующие особи, что составляет 0,6% от числа исследованных. Инфицированность животных в административных районах была равна 0,5%, городах – 1,6%, зонах отгонного животноводства – 1,1%. Степень зараженности поголовья скота варьировала от 0,01 до 4,9%. Ввиду своевременной выбраковки серопозитивных животных гематологические исследования на лейкоз не проводились. В племенном секторе носительство вируса лейкоза определили в среднем в 2,8% случаев. На 01.01.2022 в регионе было зарегистрировано 95 неблагополучных по лейкозу пунктов. За 9 месяцев 2022 г. выявили 81 новый пункт, оздоровили 18 и по состоянию на 1 октября неблагополучными официально объявлены 158 пунктов, в том числе на сельхозпредприятиях – 36 (из них 5 племхозы), в крестьянских (фермерских) – 18 и личных подсобных хозяйствах – 104. Наибольшее количество неблагополучных по лейкозу пунктов зафиксировано в Кизлярском (18), Тарумовском (17), Бабаюртовском (16), Гунибском (15), Тляратинском (10) районах и г. Махачкале (9). В Бежтинском участке, Буйнакском, Дербентском, Казбековском, Каякентском, Кизилюртовском, Хасавюртовском районах и городах Хасавюрте и Южно-Сухокумске регистрировалось по 1 очагу, в Рутульском, Унцукульском районах – по 2; в Гергебильском, Лакском, Новолакском, Цумадинском – по 3; в Сергокалинском – 4; в Чародинском – 5, в Ахвахском, Дахадаевском, Карабудахкентском – по 6; в Ботлихском, Кумторкалинском и Шамильском районах – по 7 очагов. При сравнительном

анализе серологических и молекулярно-генетических методов диагностики бычьего лейкоза установлено преимущество иммуноферментного анализа и полимеразной цепной реакции относительно применяемой в ветеринарной практике реакции иммунодиффузии.

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INTRODUCTION

Bovine leukosis (BL) is a chronic infectious disease caused by RNA-bovine leukaemia virus (BLV) belonging to *Retroviridae* family, *Deltaretrovirus* genus. The infection is widespread in many countries and remains an urgent problem in most Subjects of the Russian Federation. The numbers of diseased and infected animals have been reduced in many regions of the country owing to timely targeted preventive and elimination measures [1–3].

Losses caused by viral BL in animal industry are huge due to animal mortality rate, reduced performance, required elimination of diseased animals, destruction of animal carcasses and various organs with leukemic lesions, underproduction of young animals, costs for milk pasteurization, reduced sales of young animals, failed animal breeding activities [3–6].

According to the Information Analysis Centre of the Veterinary Surveillance Department (FGBI "ARRIAH") data, in 2021 viral BL was reported in 65 Subjects of the Russian Federation. A total of 2,070 affected localities were detected and 25 localities had remained affected since 2020. Totally 1,398.704 thousand animals were subjected to hematological examinations, 15,096 reactor animals (1.1%) were detected, 15,611 animals were sent to slaughter [7].

Since the official BL reporting (starting with the mid-60s of the last century) as a nosological disease and the use of agar gel immunodiffusion assay (AGID) for serological identification of the pathogen starting with the late 80s of the last century there has been a steady increase in the number of infected and diseased animals in the Republic of Dagestan. Therewith, numerous attempts to cope with this disease were mostly unsuccessful as the infection eradication on farms was not given due attention focusing on the eradication of such chronic diseases as brucellosis and tuberculosis [8, 9].

Clinical and hematological examinations and serological tests carried out in 1988–2017 showed high prevalence of the disease. Proportion of BLV-infected animals annually varied within 1.1–32.2% (on average – 13.3%), morbidity varied from 1.09% to 44.9% (on average – 15.3%). Proportion of BLV-seropositive animals in breeding holdings was high, 28.0% (from 6.4 to 41.5%), percentage of diseased animals was 30.6% (from 14.8 to 45.6%) [10, 11]. A small part of animals out of the cattle population was covered by routine diagnostic tests: 0.9% of animals was subjected to serological tests and 0.02% of animals was subjected to hematological examinations during the whole analyzed period [12, 13].

In line with the instruction of the Ministry of Agriculture of the Russian Federation[1], action plan[2] and draft Republican target subprogram "Prevention and elimination of bovine leukosis in holdings of the Republic of Dagestan"[3] for 2018–2020 were developed together with the Veterinary Committee experts and approved by the Ordinance of the Government of the Republic of Dagestan to ensure sustainable freedom of the region from bovine leukosis.

Effective study of the BLV infection occurrence and implementation of BL control measures in Dagestan has begun since 2018. Analysis of the disease spread dynamics for the last four years showed that the percentage of infected animals decreased and percentage of diseased animals increased. Thus, the proportion of BLV-carrier animals out of tested animals was 4.0% (proportion of diseased animals was 18.2%) in 2018; 2.9% (24.4%) in 2019; 1.4% (17.4%) in 2020 and 1.0% (19.0%) in 2021. In breeding holdings, the proportion of the virus carrier and diseased animals out of tested ones was 6.3 and 15.6%, respectively. Proportion of the cattle covered by serological tests and hematological examinations for BL in the municipal raions in 2018–2021 in dynamics was 22.9 and 0.1%; 57.4 and 0.6%; 64.8 and 0.3%; 76.2 and 0.1%, respectively [14–16].

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[1] Development of the action plan for bovine leukosis control: letter of D. Kh. Khatyov, First Deputy Minister of Agriculture of the Russian Federation No. DKh-25-27/4786 of 27 April 2016. (in Russ.)

[2] Action plan for bovine leukosis prevention and control in the Republic of Dagestan for 2017–2020 approved by the Directive of the Government of the Republic of Dagestan No. 323-r of 11 September 2017. Available at: https://docs.cntd.ru/document/450340001. (in Russ.)

[3] Amendments to the official programme of the Republic of Dagestan: "Development of agriculture and regulation of agricultural product, raw material and food product markets for 2014–2020" approved by the Ordinance of the Government of the Republic of Dagestan No. 76 of 28 June 2018. Available at: https://docs.cntd.ru/document/550147549. (in Russ.)

It should be noted that number of seropositive animals was reduced owing to expanding the test coverage of cattle in previously BL-free municipal raions located in highland, mountainous and submountaine regions of the Republic, as well as the immediate culling of AGID-positive animals without confirmation by hematological examination. Increase in morbidity is associated with long-term BL persistence in the region, small coverage of AGID-positive cattle by hematological examinations and absence of systematic targeted measures for the disease eradication.

In 2018–2021, 152 new disease-affected localities were detected, the disease was eliminated in 65 affected localities within targeted subprogram implementation in the region; 95 BL-affected localities had been officially registered by the end of 2021.

Lifetime diagnosis is the basis of anti-epizootic measures for BL. The effectiveness of lifetime diagnostic method depends on its specificity, sensitivity, easy-to-use and low cost.

Imperfect methods for diseased and virus-carrier animal detection are one of the factors impeding the reduction of a period of BL elimination in affected localities.

Two serological methods: agar gel immunodiffusion assay (AGID) and enzyme-linked immunosorbent assay (ELISA) are currently used for detection of specific antibodies to BLV at veterinary laboratories of the country. Some researchers propose using polymerase chain reaction (PCR) enabling detection of proviral DNA or viral RNA directly in blood sample taken from the virus-infected animal together with AGID and ELISA for accelerated BL elimination in holdings [17–19].

These methods are aimed at identification of the infection or presence of the disease agent in animal. Some authors propose using AGID, ELISA and PCR in combination for effective implementation of measures for BL elimination in affected holdings [20–22].

Bovine leukosis epizootic situation in municipal raions and distant pasture zones in the Republic of Dagestan was objectively assessed and comparative analysis of serological and genetic-molecular methods for BL diagnosis for their effectiveness was carried out for the first time based on the results of large-scale serological tests of cattle.

Large-scale and multifaceted study of BL epizootic features and clarification of some theoretical and practical aspects are currently required for development of complex of measures for the disease prevention and control.

The study was aimed at examination of the extent of BLV occurrence in cattle in the whole territory of the Republic of Dagestan and in its separate municipalities for selection of optimal methods for further control of BLV infection.

MATERIALS AND METHODS

The study was performed in the Caspian Regional Research Veterinary Institute – Branch of the Dagestan Agriculture Scientific Center. Official reporting data of the Veterinary Committee of the Republic of Dagestan, Republic and Raion Veterinary Laboratories for 2022 (as of 1 October) were analyzed and statistically processed for assessment of epizootic situation on BL. Cattle of different ages were tested.

AGID was mainly used for tests aimed at detection of the virus carrier animals performed at the Republic Veterinary Laboratories. Serological tests were performed in accordance with the "Methodical guidelines for bovine leukosis diagnosis"[4] and epizootological investigations were carried out in accordance with the "Methodical guidelines for epizootological investigations of bovine leukosis cases"[5].

Whole blood samples and serum samples were collected from 258 cows aged over three years and kept in BL-affected holdings located in the Gergebilsky and Gunibsky Raions for comparative assessment of the laboratory tests (AGID, ELISA and PCR) used for BL diagnosis for their sensitivity. Tests were performed using certified equipment at the GBI "Kropotkinskaya Krai Veterinary Laboratory". Serum samples were AGID and ELISA tested for bovine leukemia virus using the "Test-kit for serological diagnosis of bovine leucosis" and "Test-kit for detection of antibodies against bovine leukaemia virus with enzyme-linked immunosorbent assay (ELISA) in serum and milk (variant No. 1 – screening)" produced by the Federal State-Owned Enterprise "Kursk Biofactory" – BIOK Co. (Russia), respectively. PCR tests were performed using "FACTOR-BOVINE LEUKOSIS- PCR kit" ("VET FACTOR", Ltd., Russia).

Obtained data were statistically processed and analyzed with conventional methods [23].

RESULTS AND DISCUSSION

Positive trend for a decrease in proportion of BLV-infected animals at an increasing number of affected localities indicates that BL remains a serious problem in Dagestan. The results of diagnostic tests for BLV performed in Dagestan municipalities within the analyzed period are presented in Table 1.

A total of 632,454 susceptible animals were subjected to serological AGID tests for BL in 42 municipal raions, 3 urban districts and 7 distant pasture zones in Dagestan and 3,573 (0.6%) reactor animals were detected. Proportion of infected animals was as follows: 0.5% in administrative raions, 1.6% in urban districts and 1.1% in distant pasture zones. Proportion of affected animals varied from 0.01 to 4.9%. Totally, 70.9% of cattle population in the region were covered by serological testing. No hematological examinations for BL were carried out in the view of timely culling of seropositive animals.

Proportion of BLV-infected animals significantly varied in tested raions, towns and distant pasture zone when the epizootic situation on BLV infection was analyzed in the context of the administrative territories of Dagestan.

Currently, livestock holdings located in 12 rural raions (Agulsky, Akhtynsky, Bezhtinsky site, Gumbetovsky, Dokuzparinsky, Kazbekovsky, Kaytagsky, Kurakhsky, Magaramkentsky, Suleyman-Stalsky, Khivsky, Tsuntinsky Raions), town of Kaspiysk and administrative territories of Bakresskaya, Derbentskaya, Kochubeyskaya and Ulankholskaya distant pasture zones are free from BLV infection. Thirty rural regions, city of Makhachkala, town of Khasavyurt

Table 1
Serological tests of cattle for bovine leukosis performed in the municipalities of Dagestan in 2022 (as of October 1)

No.	Raion/town/DPZ*	Number of cattle		Number of tested animals**	Number of ADIG-positive animals (+)	
		total	cows		Number of animals	%
Raion						
1	Agulsky	10,795	3,434	4,136	–	0
2	Akushinsky	36,454	21,725	44,002	61	0.1
3	Akhvakhsky	19,531	8,686	13,060	92	0.7
4	Akhtynsky	9,778	5,585	8,993	–	0
5	Babayurtovsky	22,130	9,344	15,278	91	0.6
6	Bezhtinsky site	11,967	5,056	8,460	–	0
7	Botlikhsky	28,169	13,938	25,719	201	0.8
8	Buynaksky	43,730	12,544	20,653	24	0.1
9	Gergebilsky	21,896	9,768	13,028	52	0.4
10	Gumbetovsky	24,350	11,831	16,886	–	0
11	Gunibsky	31,506	17,488	21,668	382	1.8
12	Dakhadayevsky	27,927	12,593	16,271	77	0.5
13	Derbentsky	12,991	5,271	8,661	5	0.06
14	Dokuzparinsky	8,856	4,946	10,516	–	0
15	Kazbekovsky	13,587	6,964	10,154	–	0
16	Kaytagsky	10,439	3,754	5,808	–	0
17	Karabudakhkentsky	24,530	7,771	11,912	69	0.6
18	Kayakentsky	9,310	4,237	6,177	18	0.3
19	Kizilyurtovsky	12,730	7,569	11,969	2	0.02
20	Kizlyarsky	62,299	35,321	22,592	717	3.2
21	Kulinsky	17,089	9,299	14,141	4	0.03
22	Kumtorkalinsky	9,361	3,852	5,658	277	4.9
23	Kurakhsky	10,168	5,051	6,027	–	0
24	Laksky	30,141	12,403	24,910	38	0.2
25	Levashinsky	33,987	9,598	16,538	2	0.01
26	Magaramkentsky	18,716	8,461	18,089	–	0
27	Novolaksky	10,274	6,288	6,986	22	0.3
28	Nogaysky	22,495	16,229	15,018	3	0.02
29	Rutulsky	17,760	7,232	12,819	13	0.1
30	Sergokalinsky	9,732	4,605	7,136	56	0.8
31	Suleyman-Stalsky	11,438	9,553	13,184	–	0
32	Tabasaransky	22,376	9,661	11,497	1	0.01
33	Tarumovsky	31,171	19,236	26,335	423	1.6
34	Tlyaratinsky	8,692	3,953	15,018	3	0.02
35	Untsukulsky	19,082	9,646	11,665	89	0.8
36	Khasavyurtovsky	35,290	19,566	26,911	58	0.2
37	Khivsky	10,339	3,778	5,073	–	0
38	Khunzakhsky	24,262	11,483	13,425	11	0.1
39	Tsumadinsky	18,963	7,583	16,169	54	0.3
40	Tsuntinsky	8,439	4,403	3,203	–	0
41	Charodinsky	16,608	7,981	14,138	161	1.1
42	Shamilsky	28,192	10,967	22,125	161	0.7
City/Town						
1	Kaspiysk	887	480	575	–	0
2	Makhachkala	10,908	5,928	11,733	238	2.0
3	Khasavyurt	3,547	2,100	2,422	3	0.1
Distant pasture zone						
1	Babayurtovskaya	4,574	1,723	1,765	61	3.5
2	Bakresskaya	3,263	2,433	3,062	–	0
3	Derbentskaya	800	420	1,161	–	0
4	Kizilyurtovskaya	2,401	1,419	2,869	47	1.6
5	Kizlyarskaya	3,140	2,002	2,130	57	2.7
6	Kochubeyskaya	3,046	2,303	3,052	–	0
7	Ulankholskaya	1,756	1,095	1,677	–	0
	Total	891,872	428,556	632,454	3,573	0.6

*DPZ – distant pasture zone;
**blood samples from some cattle were retested for diagnosis confirmation.

and Babayurtovskaya, Kizilyurtovskaya and Kizlyarskaya distant pasture zones remain BLV-affected.

Single cases of BLV infection were detected in animals in the Derbentsky, Kizilyurtovsky, Kulinsky, Levashinsky, Nogaysky, Tobasaransky, Tlyaratinsky Raions and in town of Khasavyurt.

Up to 1.0% of BLV-infected animals was detected in territories of 18 administrative raions (Akushinsky, Akhvakhsky, Babayurtovsky, Botlikhsky, Buynaksky, Gergebilsky, Dakhadayevsky, Karabudakhkentsky, Kayakentsky, Laksky, Novolaksky, Rutulsky, Sergokalinsky, Untsukulsky, Khasavyurtovsky, Khunzaksky, Tsumadinsky, Shamilsky Raions).

From 1.0 to 4.9% of BLV-infected animals were reported in the Gunibsky, Kizlyarsky, Kumtorkalinsky, Tarumovsky, Charodinsky Raions, city of Makhachkala, Babayurtovskaya, Kizilyurtovskaya and Kizlyarskaya distant pasture zones.

In breeding holdings, 3.5% of dairy cattle and 0.6% of meat cattle (averagely 2.8% of the total number of tested animals) were found to be BLV-carriers. The proportion of infected animals by holding varied from 1.1 to 12.2% (Table 2). The coverage of breeding cattle with serological tests was 65.8%.

Eleven holdings were free from BLV infection, proportion of infected animals in five holdings was found to be low, from 1.1 to 3.6%. The proportion of infected animals

out of tested ones was 12.2% in two holdings only: SKhK "Agrofirma "Sogratl" (Gunibsky Raion) and AO "Kizlyaragrocomplex" (Kizlyarsky Raion).

Thirteen (72.2%) out of 18 tested breeding holdings were free from BLV infection. It should be noted that KKh "Agrofirma Chokh" and SPK Kolkhoz "Krasny partisan" where single BLV-infected animals had been detected were not declared disease-affected.

As of January 1, 2022 there were 95 BL-affected localities in Dagestan that had been initially reported in 2021. Eighty-one new leukosis-affected localities had been found for 9 months of 2022 and there were 158 reported BLV-infected localities as of the October 1, 2022: 36 agricul-

tural holdings (including 5 breeding holdings), 18 small-scale farms, 104 backyard farms.

Maximum number of affected localities was reported in the Kizlyarsky Raion (18), Tarumovsky Raion (17), Babayurtovsky Raion (16), Gunibsky Raion (15), Tlyaratinsky Raion (10) and city of Makhachkala (9). One affected locality was reported in each of the following Raions: Bezhtinsky site, Buynaksky, Derbentsky, Kazbekovsky, Kayakentsky, Kizilyurtovsky, Khasavyurtovsky and in the towns of Khasavyurt and Yuzhno-Sukhokumsk. Two affected localities were reported in each of the Rutulsky and Untsukulsky Raions; three affected localities were reported in each of the Gergebilsky, Laksky, Novolaksky and Tsumadinsky

Table 2
Serological tests of cattle for bovine leukosis in breeding holdings located in Dagestan in 2022 (as of October 1)

No.	Holding	Holding type	Number of cattle		Number of tested cattle	Number of AGID positive animals (+)		Disease status
			total	cows		number of animals	%	
Gergebilsky Raion							
1	AO "Darada-Murada"	dairy	981	645	1,809	23	1.3	affected
2	PK "Murad "	meat	1,040	627	500	16	3.2	affected
3	KFKh "Kosulya"	meat	351	243	301	–	0	free
	Gunibsky Raion							
4	SKhK "Agrofirma "Sogratl"	dairy	1,049	408	376	46	12.2	affected
5	KKh "Agrofirma Chokh"	dairy	1,302	617	614	7	1.1	free
	Dakhadaeyvsky Raion							
6	SPK "Ulluchay"	dairy	129	107	114	–	0	free
	Kizilyurtovsky Raion							
7	SPL "Agrofirma im. U. Buynakskogo"	dairy	770	450	573	–	0	free
8	OOO NPF "Plemservis"	dairy	360	338	558	20	3.6	affected
9	KFKh "Iman"	dairy	460	304	294	–	0	free
	Kizlyarsky Raion							
10	AO "Kizlyaragrocomplex"	dairy	6,159	2,762	1,578	192	12.2	affected
	Kulinsky Raion							
11	SPK "Kulinsky"	dairy	795	509	965	–	0	free
12	SPK "Plemkhoz im. B. Aminova"	dairy	257	96	273	–	0	free
	Khunzakhsky Raion							
13	SPK Kolkhoz "Krasny partisan"	dairy	380	220	520	11	2.1	free
14	SPK "Alkhas Kuli"	meat	257	110	130	–	0	free
	Shamilsky Raion							
15	SPA "Otgonnik"	dairy	285	110	280	–	0	free
16	SPK "Mesed"	dairy	356	244	628	–	0	free
	Buynaksky Raion							
17	OOO "Kurbanservis"	meat	1,504	683	1,137	–	0	free
	Kazbekovsky Raion							
18	OOO "Vympel-1"	meat	484	424	484	–	0	free
	Total		16,919	8,897	11,134	315	2.8	

Table 3
Results of diagnostic tests of cows for bovine leukosis with AGID, ELISA and PCR

Number of tested samples	Number of BLV-carrier animals detected with (number of animals/%)		
	AGID	ELISA	PCR
258	88/34.1	95/36.8	102/39.5

Raions; four affected localities were reported in the Sergokalinsky Raion; five affected localities were reported in the Charodinsky Raion; six affected localities were reported in each of the Akhvakhsky, Dakhadayevsky, Karabudakhkentsky Raions, seven affected localities were reported in each of the Botlikhsky, Kumtorkalinsky and Shamilsky Raions. Most of BL-affected localities (97.5%) were found in plain zone and other BL-affected localities (2.5%) were found in submountain zone. No BLV was found in tested animals located in highland and mountainous zones.

Bovine leukosis control is performed by governmental and regional authorities in accordance with current legal act, Order of the MOA of the Russian Federation No. 156 of 24 March 2021[6]. With putting the said Order in force the approach to BL diagnosis, declaring the localities as affected ones and elimination of the disease in BLV-affected population has significantly changed. As for disease diagnosis, the new veterinary rules provide for putting modern highly sensitive methods for early BL diagnosis (ELISA and PCR) into veterinary practice that facilitates prompt disease elimination in the affected holdings.

Results of tests of blood samples taken from cows using different methods are given in Table 3.

Comparative analysis of serological (AGID, ELISA) and molecular genetic (PCR) methods used for BL diagnosis showed the advantage of enzyme-linked immunosorbent assay and polymerase chain reaction as compared to immunodiffusion assay used in veterinary practice. Thus, 2.7 and 5.4% out of the cases previously AGID-tested negative were positive when tested with ELISA and PCR, respectively. It should be noted that all cases (100%) tested positive with ADIG were tested positive with ELISA and PCR.

"Methodical guidelines for bovine leukosis diagnosis and shortening the disease elimination period in the disease-affected holdings in the Republic of Dagestan" [24] has been developed and proposed for implementation; they provide for putting modern test methods into veterinary practice.

CONCLUSION

Analysis of data given in the paper shows that BL remains a serious problem for animal farming industry of the Republic of Dagestan as the number of affected localities especially backyard farms increases despite the positive trend for decrease in proportion of BLV-infected animals. The coverage of cattle population with diagnostic tests should be expanded for detection of all seropositive animals. Young animals at the age of 6, 12, 18 months and

animals before their introduction to main herd should be tested.

In holdings where BLV-infected susceptible animals are still detected, measures for complete disease elimination are to be taken: frequency of serological tests in the group of seronegative animals should be increased until two successive negative test results are obtained.

Complex application of AGID, ELISA and PCR as a promising test system for BL control measures is proposed for detection of maximum number of BLV-carrier animals. Putting ELISA- and PCR-based diagnosis in veterinary practice in the Republic of Dagestan will allow early detection of BLV carrier calves (sera taken from calves before first feeding with colostrum are to be tested with ELISA and sera taken from calves at the age of 15–20 days are to be tested with PCR) and enhancement of the diseases elimination effectiveness and shortening the disease elimination period at final stages of BLV infection eradication in agricultural holdings.

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