

SCREENING TESTS OF DETECTION ENZOOTIC BOVINE LEUKOSIS IN BULGARIA DURING 2016–2017

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ABSTRACT

In 2016, with blocking ELISA for antibodies in individual blood sera from cattle, seropositive reagents were found in 2 districts: Lovech - 3.8% and Montana – 35%. In 2017, the seropositive reagents in Blagoevgrad district were 17.4%. In the other 2 districts – Lovech and Sofia-city the laboratory tests were with negative result. In 2016–2017, in 3 districts a screening studies of single blood samples was carried out, for which was not perform statistically reliable results. During 2017 in NRL „EBL“ were using ELISA test for screening of antibodies in pooled and individual milk samples against gp 51. In 9 districts, 134 pooled and 3 individual milk samples were tested and a total of 101 positive reagents were detected. For control and prevention of EBL in Bulgaria, European funding is needed to implement a monitoring program.

Key words: enzootic bovine leukosis (EBL), screening, ELISA.

Introduction

Enzootic bovine leucosis (EBL) is an infectious lymphoproliferative disease, spread throughout the world, USA, Europe, and in our country. The economic importance of the disease is determined by a reduction in milk production of over 50%, premature scrapping of valuable animals and loss of fertility. EBL is caused by exogenous delta virus and is characterized by a persistent infection in the subpopulation of B lymphocytes. The proviral DNA interferes with cellular DNA. The disease spreads horizontally through transfer of blood or secretions containing infected lymphocytes. The productive animals can be infected at any age including the embryonic stage. Most cattle are in inactive form, ~ 30% over 3 years of age have persistent lymphocytosis and / or lymphoblastosis. In a lesser extend 0.1% to 10% develop lymphosarcomas (tumors) in the internal organs (OIE, Manual, 2012). Some studies conducted earlier in Bulgaria in 1998-2000 show a wide spread of EBL in 5 regions – Burgas, Stara Zagora, Sliven, Yambol and Haskovo. By serological examinations of 200,518 individual blood serum samples with an agar gel immunodiffusion test (AGID) using the Ouchterlony method, modified by Miller and van der Maaten (1976), the highest percentage of EBL in Burgas region was found – 33.07 % at mean percent for other areas – 17.02% (Sandev et al., 2001). Serological studies conducted at a later stage over a 5-year period (2005–2009) through an AGID test showed a consistently high rate of positive reagents in the country ranging from 14.46 % to 23.97 % at an average – 20,16% (Sandev et al., 2010). In 2012, the serological analysis of EBL prevalence in 28 regions shows an average rate of positive reagents of 33.38 %, ranging from 13.05% to 63.85% (Sandev et al., 2015).

The aim of this study was to present the laboratory data from the serological screening of EBL for the period 2016–2017 in order to highlight the need for funding (European or national) and to approve a monitoring program for control and prevention of the disease in Bulgaria.

Materials and methods

The data from the diagnostic tests carried out in the NRL "EBL" of the tested blood and milk samples (pooled and individual) received according Article 118 of the Bulgarian Veterinary Legislation

and paid tests during the last two years have been used. Screening of blood serum from cattle in 2016 was performed with blocking ELISA (IDEXX, Montpellier, SAS, France) against gp 51 of EBL.

In 2016–2017, an ELISA screening method (IDEXX, Leukosis Milk Screening, France) was accredited and established for the detection of antibodies in milk samples (pooled and individual) from cattle. The tests were performed with the two ELISA kits according to the standard operating procedures described in OIE Manual, 2012. For this purpose, an ELISA system model Zenith with 340 nm up to 450 nm wavelength, (Antos Labtec Instruments GmbH Salzburg, Austria) and a washing machine Elx 50 (Bio-tec Instruments, Inc.) were used.

Results and discussion

The results of the screening investigations in 2016–2017 for the detection of EBL are presented in (Table 1).

Table 1: Screening tests of detection EBL in 2016 2017

Samples type/ Test of study	Year	Regional Food Safety Agency/ total number of tested samples	Positive samples	Negative samples	
Blood serums ELISA blocking	2016	Lovech`	104	4	100
		Montana	20	7	13
		Plovdiv	1	-	1
		Smolyan	2	-	2
		Sofia distict	1	-	1
		Sliven	2	2	-
		Total :	130	13	117
Blood serums ELISA blocking	2017	Blagoevgrad	23	4	19
		Lovech	39	-	39
		Dobrich	5	2	3
		Turgovishte	5	1	4
		Sofia-distict	1	-	1
		Sofia-town	10	-	10
		Total :	83	7	76
Milk samples ELISA screening	2017	Blagoevgrad	16	7	9
		Vidin	15	10	5
		Vratza	16	14	2
		Lovech	16	13	3
		Kyustendil	12	8	4
		Montana	13	10	3
		Pernik	16	12	4
		Pleven	12	7	5
		Sofia-town	15	15	-
Sofia-distict	6	5	1		
		Total:	137	101	36

In 2016, a total of 130 blood serum samples were received from the 6 Regional Food Safety Directorate (RFSD) in the NRL. By a Blocking ELISA 13 positive reagents were detected and 117 samples were with a negative result. The largest number of samples were tested from the Lovech region – 104 blood serum and 4 (3.8%) positive reagents were found; from the Montana region in 20 blood serum 7 (35%) were confirmed as positive reagents and in Sliven region 2 positive results. It can be noted that from 3 districts – Plovdiv, Smolyan and Sofia district only single samples were received and a negative result was reported. In 2017, by a Blocking ELISA 83 samples from 6 RFSD were tested and 76 samples were negative. In 3 districts – Blagoevgrad, Dobrich and Turgovishte, 7 positive reagents were detected. The small number of samples from the last 2 regions cannot give statistically reliable results. When interpreting the data, all tested blood samples with a S / N ratio (%) $\leq 40\%$ were reported as positive for BLV antibodies, respectively those with S / N (%) $\geq 40\%$

were considered negative. In 2017, a total of 134 tank milk samples were delivered from 10 RFSD – Blagoevgrad, Vidin, Vratsa, Lovech, Montana, Pernik, Pleven, Sofia district, Kyustendil, and three individual milk samples. The samples were tested with the ELISA screening test and 101 positive results were found and 36 of them were negative. Positive samples were considered at ratio at $\geq 70\%$ and at ratio of $\leq 60\%$ reported as negative. Samples with a percentage between $> 60\%$ and $< 70\%$ were considered inconclusive and were retested. After establishing a positive result in pooled milk samples, it was recommended to send additional samples from the same RFSD for ELISA Verification Test, accredited in the NRL from November 2017. The serological tests were performed with commercial ELISA kits (blocking and screening), which included standard reagents approved and certified in the European Union for detecting BVL antibodies. The 2016–2017 summary data showed that 20 (~ 9.39%) seropositive reagents were found in 213 blood samples and in 134 pooled and 3 individual milk samples, the positive results were 101 at the average ratio of 73.7% which outlines the clear trend that the infection is spread in Bulgaria. Previous studies by some authors (Sandev et al., 2001; 2010; 2015), as well as recent data, confirm the presence of the BLV in a high percentage of EBL. This shows the need to encourage the investigation of a larger number of blood serums and milk samples to be tested in NRL for screening by individual regions. In order to establish the prevalence of EBL at this stage, a monitoring program have to be funded in the country. The identification of infected animals from farms will help to limit the viral infection and prevent Bulgaria from EBL.

Conclusion

In 6 districts – Montana, Lovech, Blagoevgrad, Sliven and Dobrich in 2016-2017 20 positive reagents were detected in ELISA with the average rate of 9.39%.

When tested pooled and individual milk samples with ELISA screening test through 2017 in 9 districts – Sofia-city, Vratsa, Lovech, Pernik, Montana, Vidin, Blagoevgrad, Pleven and Sofia-region were shown 101 positive results at an average rate for these areas – 73.72%.

The reported data on the prevalence of EBL in the country show that it is necessary to approve and finance a national monitoring program control and prevention of the disease.

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