Slov Vet Res 2012; 49 (2): 79-87
UDC 636.2-09:616-002.5:57.083.3:616-036.22

Original Scientific Article

BOVINE TUBERCULOSIS IN CATTLE DURING THE IMPLEMENTATION OF OFFICIAL CONTROL MEASURES IN REPUBLIC OF MACEDONIA FOR THE PERIOD 2007-2009

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Summary: Tuberculosis in cattle has been subject of different control programmes since late 40's of the last century. Latest *Multi-Annual National Programme for Eradication of Bovine Tuberculosis in Cattle in Republic of Macedonia* has been adopted and started implementing in 2007. This study is an evaluation of the results from implementation of this program.
A retrospective and descriptive study has been carried out. Demographic and epidemiological characteristics of the bovine tuberculosis were evaluated. An average of 160 784 (61.96%) of total number of cattle were covered by this programme. The single tuberculin test (STT) was positive in 1 021 (0.63%) of the tested animals. Only 952 (93.21%) animals that reacted positive to the single tuberculin test were subjected to comparative tuberculin testing, and an average of 390 (40.95%) of them were declared as positive. In 2007, 43 573 herds (holdings) were tested for bovine tuberculosis, where 173 were found to be positive; in 2008 from 43 753 tested herds, 253 were positive and in 2009 from 42 714 tested, 265 were positive. All animals found positive for bovine tuberculosis were slaughtered in the sanitary slaughterhouse and the milk was declared as not fit for human consumption.
Regarding bovine tuberculosis, Republic of Macedonia is considered as country with low prevalence with overall average prevalence of 0.002% for the period 2007-2009. The North-west, South-west and Eastern regions of the country were more intensively affected by the disease. For more efficient control, the number of tested cattle should be increased, followed by increasing of the sanitary measures and epidemiological tracing–back.

Key words: tuberculosis; cattle; tuberculin test; zoonosis; food safety

Introduction

Bovine tuberculosis (bovine TB) is a chronic bacterial disease of animals and humans caused *by M. bovis and M. caprae*. Large number of countries are reporting bovine TB as a major infectious disease among cattle, other domestic animals and certain wild animals. Transmission to humans is major public health issue [1]. Bovine TB causes severe economic losses in livestock due to loss of production, mortality, and condemnation of carcasses [2].

Received: 17 October 2011
Accepted for publication: 30 March 2012

In 1882, the time of Koch's discovery of the etiological agent, between 20% and 40% of the cattle in many European countries had tuberculosis [3].

Developed countries, control bovine TB by testing and slaughtering the animals, compulsory pasteurization of milk and minimizing the risks for human infections. In those countries, clinical cases of tuberculosis in cattle are seldom occurring due to the programmes for control and eradication, which enables early detection and presumptive diagnosis with consecutive elimination of infected animals before appearance of clinical signs [4]. Control programmes in western European countries were established as far as the 1930-s [5], and in central European countries in

the 1960-s [6]. Sweden declared it's officially tuberculosis free status in 1958 [7], USA in 1991 even though the eradication campaign started in 1917 [8]. In Canada the disease was brought under the mantle of official notification and eradication program in 1923. All Canadian cattle herds that were not under quarantine were recognized as being TB-free in 1997 [9].

Bovine TB is present in the Balkan region. According to the OIE, the average prevalence in Albania for the period 2007-2008 was 0.018%, in Serbia for the period 2007-2009 was 0.006%, and in Greece for the same period reported average prevalence of 0.052% [10]. The results are provided in Table 1.

Table 1: Cattle population and bovine TB in four Balkan countries according to OIE *

	Cattle population (No. of)			Bovine TB (No. of)					
Year	Country	Anim.	Establish.	New outbr.	Susceptib. Anim.	Cases	Deaths	Destr.	Slaugh
2007	Albania	634 000	256 356	18	71 037	113	0	0	113
	Bulgaria	582 594	199 610	0	0	0	0	0	0
	Greece	880 110	32 674	42	2 921	328	17	0	1 664
	Serbia	1 735 248	299 264	36	256	63	0	0	7
2008	Albania	699 502	271 230	15	35 332	137	0	0	97
	Bulgaria	584 468	195 071	2	2	2	0	0	1
	Greece	893 046	35 250	38	2 835	438	0	nr	121
	Serbia	1 057 000	305 647	35	764	94	0	1	58
2009	Albania	541	226 443	7	2 890	54	0	0	54
	Bulgaria	584 283	127 060	0	0	0	0	0	0
	Greece	749 623	38 041	48	3 824	525	3	0	222
	Serbia	1 087 504	21 5226	32	857	73	0	0	54

nr = not reported; anim. = animals; outbr=outbreaks; suscept=susceptible; destr=destroyed;slaugh.=slaughtered
*There are no data for Kosovo and Montenegro

Bovine TB infection in cattle is usually diagnosed in live animals on the basis of delayed hypersensitivity reactions. The World Organization for Animal Health (OIE) prescribes the tuberculin test (TT) as a reliable method for screening of bovine TB [1]. It involves measuring skin thickness before and after tuberculin is injected into the measured area. The single tuberculin test (STT) uses bovine tuberculin, while comparative tuberculin test (CTT) uses both, bovine and avian tuberculin. The second test is used mainly to differentiate between animals infected with bovine TB and those sensitized to tuberculin due to exposure to other mycobacteria or related general. The TT is used generally and depends on the prevalence of tuberculosis infection and on the level of environmental exposure to the other sensitizing organisms among animals evaluated. The reactions are interpreted on the basis of appropriate schemes [1].

The objective of this study was to analyze the epidemiological situation with bovine TB in the cattle in Macedonia for the period from 1 January 2007 to 31 December 2009 and to assess the efficiency of the bovine TB eradication programme in cattle population.

Material and methods

Animals and herds

The territory of Republic of Macedonia is divided into 84 epidemiological units which correspond to administrative and territorial division of the coun-

try. The system for identification and registration (I&R) of cattle in Republic of Macedonia regulates holdings as equivalent to herds due to the large number of small farms (herds) by average of 5.99 cattle per holding during the evaluation period. The Multi-Annual Programme for Eradication of Bovine TB requires record keeping for all cattle together, not by different categories. All bovine animals older than 6 weeks were subject to testing. STT was performed by the veterinary stations contracted by the Food and Veterinary Agency (FVA). Based on their reports, STT positive animals were submitted for CTT. The CTT was performed by the Faculty of Veterinary Medicine-Skopje.

Skin testing

The STT involved intra-dermal injection of 0.1 ml of 2000 International Units (IU) of bovine purified protein derivate tuberculin (B-PPD) (Veterina, Croatia) in the mid-neck of the animal. Firstly, the mid-neck section was clipped with scissors and then skin-fold thickness was measured with kutimeter (calipers). The injection of B-PPD was administered via intra-dermal route in the measured place and the skin-fold thickness was measured after 72 hours. The STT reaction was considered positive when clinical signs (diffuse or extensive edema, exudation, necrosis, pain or inflammation of the lymphatic ducts in that region or of the lymph nodes) were detected or increase of 4 mm or more in skin-fold thickness. Inconclusive reaction was considered when the increase in skin-fold thickness was more than 2 mm and less than 4 mm without manifestation of aforementioned clinical signs. Animals with inconclusive or positive reactions by the STT were subjected to a CTT (re-testing) after 42 days interval as desensitization period. The test was carried out by intradermal injection of 0.1 ml of 2000 IU of B-PPD (Veterina, Croatia) in the mid-neck of the animal, by simultaneous intra-dermal injection of 0,1 of 2000 IU of avian PPD tuberculin (A-PPD) (Veterina, Croatia) at a distance of 12-15 cm from the first one. Animals with increased skin-fold thickness at the bovine site of injection higher than 4 mm than the reaction at the avian injection site were declared as infected with causal agent of bovine TB. The CTT and the interpretation of the results were performed by the Faculty of Veterinary Medicine - Skopje [1, 11].

Reactors' management

According to the *Multi-Annual National Programme for Eradication of Bovine Tuberculosis in Cattle in Republic of Macedonia*, all cattle found positive by CTT were removed from the herd and slaughtered in sanitary slaughterhouse within a period of maximum one month. The movement and trade of the other bovine animals from the infected herds, has been prohibited. The animal holdings were cleaned and disinfected. All farmers, whose cattle were slaughtered, received reimbursement funds by the FVA. The milk of the suspected and infected animals was declared as non-fit for human consumption. Following the *post-mortem* inspection, the meat from slaughtered cattle was used in the meat processing industry following a heat treatment ensuring destruction of the bacillus. In cases of generalized form of TB, the cattle corpses were confiscated and safely disposed [11].

Results

In all 84 epidemiological units STT was performed on 60.9% in 2007, 69.4% in 2008 and 55.5% in 2009 from all bovine animals. The STT was positive in 0.50%, 0.70% and 0.71% of all bovine animals subjected to STT in 2007, 2008 and 2009 respectively. Out of all STT positive reactors, an average of 92.85% were re-tested with CTT (90.51% in 2007, 95.37% in 2008 and 92.67% in 2009: Table 2). In comparison to 2007, a significant increase of 39% of CTT re-tests were performed in 2008. Furthermore, there were total 30% more positive animals in 2008 in comparison to 2007. A decline of 22% in the total performed CTT re-tests was observed in 2009, with total 13% less positive animals than in 2008.

Table 2: Results from the 2007 to 2009 National eradication program

	Years			Averages
	2007	2008	2009	2007 to 2009
Total No of cattle	261 058	259 479	257 900	259 479
Total No STT tested	158 965	180 150	143 237	160 784
% of STT tested (from all cattle)	60.89%	69.43%	55.54%	61.95%
Total No STT positive	801	1 254	1 010	1 021
% of STT positive (from STT tested)	0.50%	0.70%	0.71%	0.64%
Total No CTT tested	725	1 196	936	952
% CTT tested (from STT positive)	90.51%	95.37%	92.67%	92.85%
Total No CTT positive	319	454	396	390
%CTT positive (from CTT tested)	44%	37.96%	42.31%	41.42%

During the evaluation period of this study, animals positive on bovine TB were detected in 38 epidemiological units (45.23% of all units). The disease was not confirmed in the central-west and south-east parts of the country. The presence of the disease was confirmed constantly in 20 epidemiological units in the north-west, south-west and the eastern part of the country. In 5 epidemiological units: Vinica, Gostivar, Probistip, Sveti Nikole and Struga, the disease was confirmed for the first time in 2008 and was present also in 2009. Those epidemiological units are situated in the north-west, east and the south-west part of the country. Despite the newly confirmed bovine TB cases in 2008, in 2009 the number of epidemiological units affected by the disease decreased from 19 to 13. Furthermore, in 2009 there were no newly confirmed cases of bovine TB in the epidemiological units that were declared as free from TB in the previous years (Table 3).

Geographical presentation of the presence of the bovine TB in different areas is given in fig. 1, 2 and 3, where the red triangles stand for the epidemiological units affected with the disease.

The average point prevalence of bovine TB among the tested animals in the evaluation period was 0.002% (0.0020% in 2007, 0.0025% in 2008 and 0.0027% in 2009).

Figure 1: Bovine tuberculosis occurrence in 2007

Table 3: Bovine TB positive epidemiological units, animals and herds

epidemiological units where CTT was performed	Bovine TB in 2007			Bovine TB in 2008			Bovine TB in 2009		
	CTT animals tested	CTT Positive animals	No. of positive herds	CTT animals tested	CTT Positive animals	No. of positive herds	CTT animals tested	CTT Positive animals	No. of positive herds
Bitola	43	9	5	79	7	5	48	9	7
Mogila	19	1	1	28	4	2	43	5	4
Novaci	20	2	1	31	5	3	29	2	2
Veles	7	0	0	2	0	0	0	0	0
Vinica	2	0	0	4	1	1	4	1	1
Vrapciste	43	8	3	10	2	2	6	4	4
Gostivar	0	0	0	18	7	6	26	6	6
Delcevo	0	0	0	2	0	0	0	0	0
Kamenica	33	0	0	11	0	0	14	0	0
Rosoman	0	0	0	1	0	0	0	0	0
Kichevo	0	0	0	4	0	0	0	0	0
Kochani	19	1	1	3	1	1	12	2	2
Kratovo	9	1	1	8	3	2	11	4	1
Kr. Palanka	0	0	0	1	0	0	0	0	0
Kumanovo	13	3	2	18	2	2	26	12	7
Lipkovo	13	2	2	26	4	3	37	11	8
Nagorichane	21	9	4	19	4	2	18	4	3
Ohrid	0	0	0	5	0	0	0	0	0
Prilep	0	0	0	3	0	0	4	0	0
Probishtip	0	0	0	3	1	1	4	1	1
Konche	4	0	0	0	0	0	0	0	0
Radovish	7	0	0	0	0	0	9	0	0
Resen	0	0	0	1	0	0	0	0	0
Lozovo	0	0	0	3	0	0	0	0	0
Sveti Nikole	14	0	0	9	4	3	7	2	2
Ilinden	22	10	2	7	4	2	24	2	2
Petrovec	3	1	1	8	1	1	9	1	1
Gazi Baba	3	1	1	4	2	2	9	3	2
Saraj	8	3	2	47	22	9	71	46	21
Struga	0	0	0	6	2	2	19	2	2
Brvenica	80	66	17	58	25	16	96	41	32
Zelino	76	52	43	327	143	86	147	68	53
Jegunovce	4	1	1	8	4	3	10	4	4
Bogovine	55	31	17	127	57	27	43	14	8
Tearce	22	9	7	68	31	23	34	22	17
Tetovo	180	108	61	248	115	47	164	129	74
Karbinci	0	0	0	2	0	0	1	0	0
Shtip	5	1	1	7	3	2	11	1	1
Total	725	319	173	1 196	454	253	936	396	265

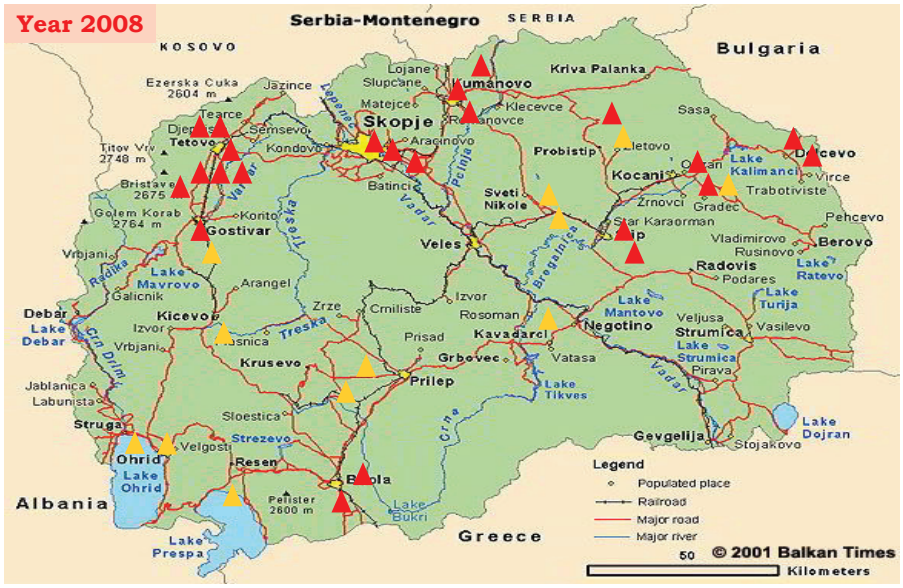

Figure 2: Occurrence of bovine tuberculosis in 2008. Epidemiological units first time suspected are Gostivar, Vinica, Rosoman, Kicevo, Debarca, Dolneni, Krivogastani, Probistip, Resen, Sveti Nikole, Lozovo, and Struga (yellow triangle)

Figure 3: Bovine tuberculosis occurrence in 2009

Discussion

Since 1940-s, many different strategies and programmes for control and eradication of bovine TB have been carried out in Republic of Macedonia. The latest *Multi-Annual Program for Eradication of Bovine Tuberculosis* was adopted in 2007. Before the starting of this program, during the period of 2004-2006 STT was performed on 39.2% of the cattle population with prevalence of 0.045% [12]. According to our results for the first three years (2007-2009) of the implementation of the latest programme, the percentage of STT performance increased up to 61.95% with average prevalence of 0.002% of the cattle population.

For the evaluation period, out of total 84 epidemiological units, TB positive reactors were found in 38 (45.23%), where 20 (23.81%) of them were continuously giving positive reactors.

In 2007, 173 (0.4%) holdings out of 43 573 were positive in 20 epidemiological units. In 2008, 253 (0.58%) holdings out of total number of 43 753 were positive where 240 (94.86%) of the positive holdings originated from 20 continuously infected epidemiological units. In 2009, out of 42 714 total holdings, 265 (0.62%) were positive, with 253 (95.47%) originating from 20 continuously infected epidemiological units.

If we analyze the distribution of individual positive animals, all 319 positive reactors were from 20 continuously infected epidemiological units in 2007. In 2008, 439 (96.7%) out of 454 positive reactors were detected in the same 20 continuously infected epidemiological units. In 2009, 384 (97.7%) out of 393 positive reactors originated from the same 20 continuously infected epidemiological units.

The results of this study show lower prevalence of TB than the results given by various authors from other world regions: Arab peninsula (0.12%) [13]; Africa (from 0.55% to 2.1%) [14, 15, 16]; Ecuador (3.85%) and Uruguay (0.5%) [2, 17].

By analysis of the comparison of the reports submitted to OIE, it can be noted that the average prevalence of bovine TB in the neighboring countries for the same period (2007-2009) is similar (Albania 0.018%, Serbia 0.006% and Greece 0.052%). Bulgaria has reported only 2 cases of bovine TB in 2008 (0.0003%) while there are no reports from Montenegro and Kosovo [10]. The missing data from Kosovo is particularly important having in mind bordering North-West region of Macedonia with highest prevalence of TB (Table 3. the epidemiological units of Brvenica, Zelino, Tearce, Jegunovce, Bogovinje and Tetovo). *Ameni and al.* detected the lack of quarantine and smuggling of live animals across borders, as factors that promote transmission of *M. bovis* from one country to another, as well as persistent infections in such regions [18].

With exception of Greece with 23.9 animals per holding, average holding (herd) size in rest of the neighboring countries is similar to Macedonian 5.9 (Albania 2.5, Bulgaria 3.5 and Serbia 4.7) [10]. *Perez et al.* suggest that herd size play important role in spreading of TB where large farms are at higher risk than small farms [2]. Our data indicate the opposite, finding positive reactors mostly in the small holdings. This can be supported by average 1.84; 1.79 and 1.48 positive animals per infected holding for 2007/08/09 respectively.

In this study STT positive reactors were found in 0.64% of the total tested cattle, while the CTT confirmed only 41.4% of positive STT. In a survey conducted in a country with higher prevalence of the bovine BTB, a total of 4.24% of the cattle were positive to the STT and 88.6% of them were positive to the CTT [2].

The CTT was not performed in approximately 7% of the STT positive and inconclusive animals. Those animals were dead, slaughtered or sold. In other studies, awareness of the breeders for the disease was detected as a crucial factor for the eradication of the disease. The level of disease awareness among famers was related to the prevalence of the disease, the higher the prevalence of the disease is, the higher is the awareness [19, 20]. This high percentage of animals escaping the retesting with CTT has been identified as a problem where the competent authority should focus its attention to stricter implementation of the movement control measures in STT positive holdings.

Conclusions

A period of three years (2007-2009) is insufficient for full evaluation of the efficiency and effectiveness of the *Multi-Annual National Programme for Eradication of Bovine Tuberculosis in Cattle in Republic of Macedonia.* Nevertheless it is providing some preliminary data that can be used as direction of further improvement and adjustment of the programme. By means of the implementation of the program in the first three years, the total number of tested animals has been increased giving better information for the prevalence of bovine TB in the country, distribution of infected holdings and their grouping in epidemiological units.

In general, Republic of Macedonia can be considered as a low prevalence country for bovine TB in cattle. The North-West region of the country has the highest prevalence of bovine TB. The major reasons for persistence of bovine TB in this area are insufficient movement control, incomplete depopulation of infected herds and deficient disinfection. In this part of the country, cooperation with cattle owners is also critical. For the successful implementation of TB control programme, public awareness on the importance of tuberculosis as an animal and public health risk should be raised on a higher level, particularly for the cattle farmers.

In Republic of Macedonia, bovine TB is present dominantly in small holdings, grouped in traditional areas of 20 epidemiological units. The main reasons for persistence of the disease in those holdings are weak movement control, lack of biosafety measures and joint pasture. The eradication programme should be extended also to the wild animals on the whole territory of the Republic of Macedonia, especially along the borders with Albania, Kosovo and Serbia.

The lack of data on the situation with Bovine TB in Kosovo and Montenegro must be overcome by bilateral data exchange between the veterinary authorities of those countries or by providing their reports through OIE.

The eradication programme for bovine TB should engage other diagnostic methods than TT. Molecular biology tests such as PCR as well as bacteriology must accompany STT and CTT. Low percentage of confirmation between STT and CTT (41.4%) as well as many inconclusive cases (e.g. generalized TB, cross-reactive animals etc.) can be resolved by those methods.

Government should provide stabile financing of the Program for control and eradication of tuberculosis, particularly for the timely reimbursement to the farmers and fast removal of the positive reactors from the holdings as well as for the implementation of the measures prescribed by the programme.

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TUBERKULOZA PRI GOVEDU V ČASU UVAJANJA URADNIH UKREPOV NADZORA V REPUBLIKI MAKEDONIJI OD LETA 2007 DO 2009

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Povzetek: Tuberkuloza pri govedu je bila predmet različnih programov nadzora od konca 40-ih let prejšnjega stoletja. Zadnji večletni nacionalni program za izkoreninjenje tuberkuloze pri govedu v Republiki Makedoniji je bil sprejet 2007 in se je istega leta začel izvajati. Članek povzema raziskavo vrednotenja rezultatov izvajanja tega programa. Izvedeni sta bili retrospektivna in opisna študija. Ocenjene so bile demografske in epidemiološke značilnosti tuberkuloze pri govedu. V programu je bilo zajetih 160784 ali 61,96 % vseh govedi. Enotni tuberkulinski test (STT) je bil pozitiven v 1021 primerih ali pri 0,63 % testiranih živali. Le 952 živali (93,21 %), ki so se odzvale pozitivno na enotni tuberkulinski test, je bilo vključenih v primerjalno tuberkulinsko testiranje, od tega jih je bilo 390 (40,95 %) pozitivnih. Leta 2007 je bilo na govejo tuberkulozo testiranih 43573 čred (gospodarstev), kjer je bilo odkrito 173 pozitivnih, v letu 2008 je bilo od 43753 testiranih čred 253 pozitivnih in v letu 2009 od 42714 testiranih 265 pozitivnih. Vse živali, ki so bile pozitivne na govejo tuberkulozo, so bile zaklane v sanitarni klavnici, njihovo mleko pa je bilo označeno kot neprimerno za prehrano ljudi. Republika Makedonija spada med države z nizko stopnjo prevalence tuberkuloze pri govedu s skupno povprečno prevalenco 0,002 % v obdobju 2007-2009. Severozahodna, jugozahodna in vzhodna področja države je bolezen močneje prizadela. Za učinkovitejši nadzor je potrebno povečati število testiranih goved, čemur morajo slediti sanitarni ukrepi in epidemiološko sledenje.

Ključne besede: tuberkuloza; govedo; tuberkulinski test; zoonoza; varnost hrane