EPIDEMIOLOGICAL CHARACTERISTICS OF BRUCELLOSIS IN SHEEP AND GOATS IN BULGARIA: 2005–2008

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Abstract: Aim: To analyse and present the epidemiological and epizootological situation of brucellosis in humans, sheep and goats caused by *Brucella melitensis*.

Methods: The veterinary health services analyzsd epidemiological reports, as well as other official documents, in relation to the epidemiology and the measures undertaken for control of brucellosis in Bulgaria, 2005–08.

Results: In Bulgaria animal infections caused by *Brucella melitensis* have been eradicated since 1941. In 2005 several Bulgarian citizens with *Brucella* infection were diagnosed in the National Reference Laboratory. All the cases were imported, mostly by employees on sheep and goat farms in Greece. For a period of four years, the number of infected humans was 120 and distributed in 12 districts. Epidemiological investigations suggested that 45 persons (37.5%) were infected in Greece and 4 in Cyprus, Turkey, Italy and Tanzania respectively. All other patients were considered to be infected in Bulgaria. They were owners of infected sheep and goats or consumers of contaminated dairy products with *Brucella melitensis*. In June 2006, the first outbreaks of brucellosis in sheep and goat farms were recorded in the Smolyan district. By the end of 2008, the infected herds was 12.2% in goats and 1.8% in sheep. In order to achieve eradication 496 goats and 117 sheep were slaughtered.

Conclusion: A farm where goats from Greece were introduced illegally in 2005 was identified as the primary source of the infection. the negative epidemiological aspects of free movement of goods and persons in the European Union are considered.

Key words: *Brucella melitensis*, brucellosis in humans, brucellosis in sheep and goats, emerging zoonosis.

Introduction

The causative agent of brucellosis in sheep and goats was discovered in 1887 on the island of Malta [1]. This zoonosis, known also as Malta or Mediterranean fever, is widespread throughout the world. Mediterranean countries are considered as a primary endemic region [2]. Regardless of carrying out regulated EU legislative measures the disease is distributed mainly in the Southern Europe Member States bordering the Mediterranean Sea (Portugal, Spain, Italy, Greece and Turkey [3]. On the Balkan Peninsula, Brucellosis in sheep and goats is widespread in Macedonia, Albania and Bosnia and Herzegovina. The situation is similar in Georgia, Armenia and Azerbaijan. From 1941 until the middle of 2006, Bulgaria was considered free of animal brucellosis caused by Brucella melitensis. In 1947 an epizootic outbreak was diagnosed in a herd of Karakul breed sheep imported from abroad [4]. In 2005, 37 human cases were diagnosed, all of them imported, and in 2006 the first outbreaks of this zoonosis were diagnosed in sheep and goat herds in the Smolyan and Yambol districts [3, 5]. Data obtained following epidemiological and epizootological investigations of brucellosis in humans, sheep and goats caused by B. melitensis in Bulgaria for a four-year period are presented.

Methods

Assessments of the prevalence and distribution of brucellosis in sheep and goats in animals and in humans were performed according to the official reporting of infectious diseases from the National Veterinary Service (NVS) and the National Centre for Infectious and Parasitic Diseases (NCIPD) for 2005–08. In the affected regions, epidemiological and epizootological investigations were conducted for identification of the source of infection and modes of transmission. For the analysis of the distribution of brucellosis in sheep and goats the EU Member States we used the Annual Reports on trends and sources of zoonoses and food-borne infections of the European Food Safety Authority [6, 7].

Results

Brucellosis in animals

Table 1 and Figure 1 present data on the prevalence and geographical distribution of brucellosis in sheep and goats in Bulgaria for the period 7.6.2006 to 31.12.2008. The disease was registered in 16 villages of 4 districts: Smolyan (2 villages), Yambol (1), Haskovo (11) and Stara Zagora (2). Epidemiological

investigations concluded that brucellosis of sheep and goats occurred initially in a herd of goats reared in the suburbs of Harmanli, Haskovo region. This probably occurred in 2005 with goats moved from Greece without veterinary certificates. Serological tests in this district established that the incidence rate in affected herds was 12.2% in goats, and 1.8% in sheep. At the end of 2005, the infected goat herd was sold in 11 villages of 3 districts without veterinary certificates.

Table 1 – Табела 1

Prevalence of brucellosis in sheep and goats in Bulgaria: 7.VI. 2006–31.XII. 2008
Преваленција на бруцелозаша кај овци и кози во Бугарија:
7. VI. 2006–31.XII. 2008

Affected villages	Date of establishment/ number of susceptible animals in the affected village	Number of infected and killed goats/ % of infected goats	Number of infected and killed sheep/ % of infected sheep	Source of infection
1. Zlatograd, Smolian district	7.6.2006 199 goats (g) 425 sheep (s) 264 cattle	3/1.5		Purchase of 2 goats without a certificate from Harmanli
2. Startsevo, Zlatograd municipality, Smolian district	22.6.2006 49 g 61 s 274 cattle	2/4.1		Purchase of 2 goats without a certificate from Harmanli
3. Straldja, Yambol district	<u>17.10.2006</u> 209 g 502 s	4/1.9	2/0.4	Not established
4.Valche Pole, Lubimets municipality, Haskovo disrtict	20.8.2007 271 g 377 s	68\25.0 (1 male goat)	66\17.5 (1 ram)	Purchase of 2 goats without a certificate from Harmanli
5. Harmanli, Haskovo district	11.9.2007 319 g 550 s 440 cattle	87/27.3	26/4.7	Probably some goats of Greece origin moved without certi- ficate in a herd situa- ted in cottage zone of Harmani in 2005
6. Dripchevo Harmanli municipality, Haskovo district	21.9.2007 217 g 280 s 1 cattle	4/1.8		Purchase of 1 goat without a certificate from Harmanli
7. Nadezden Harmanli municipality, Haskovo district	4.10. 2007 291 g 279 s 279 cattle	2/0.7		Purchase of 1 goat without a certificate from Harmanli
8. Levka Svilengrad municipality, Haskovo district	<u>4.10. 2007</u> 543 g 450 s <u>280 cattle</u>	3/0.6		Purchase of goats without a certificate from Harmanli

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9. Dostievo	4.10. 2007			Purchase of goats
Harmanli	235 g			without a certificate
municipality,	480 s	1/0.4		from Harmanli
Haskovo district	35 cattle			
10. Cherna	4.10. 2007			Purchase of 13 goats
Mogila	268 g	234/87.3		and one male goat
Lubimets	29 s	229 goats I		without a certificate
municipality,	5 cattle	one herd		from Harmanli
Haskovo district				
11. Ivanovo	12.10.2007			The cow was
Harmanli	156 g			purchased from
municipality,	950 s			Harmanly
Haskovo district	478 cattle			
12. Ustem	23.10.2007			Common water pond
Harmanli	630 g			with animals from
municipality,	1100 s	1/0.2		Mramor
Haskovo district	502 cattle			
13. Mramor	5.12.2007			Probably goats from
Topolovgrad	414 g			Harmanli
municipality,	334 s	85/20.5	21/6.3	
Haskovo district	115 cattle			
14. Opan	19.9.2007			The goat was
Stara Zagora	65 g			purchased from
district	126 s	1/0.8		Harmanli
	60 cattle	_,		
15. Vasil Levski	10.11.2007			One sheep
Stara Zagora	62 g		2/0.7	originating from
district	296 s			Harmanly
	104 cattle			
16. Balgarin	1.6.2008			One goat purchased
Haskovo district	121 g			from Harmanly
	324 s	1/0.8		
	160 cattle			

In Ivanovo (Haskovo district) a cow purchased from Harmanli was found to be infected. In the village of Ustrem, positive goats were infected from a watering pond shared with animals from Mramor. In one village (Straldzha,Yambol region) the source of infection was not established. Seven positive cows were found in the following villages: Harmanli (3), Straldja (1) and Mramor (3). In affected herds, the prevalence rate in goats was 7 times greater than in sheep, an average 12.2% and 1.8% respectively. The highest rate of infected goats was registered in Cherna Mogila (87.3%) which was because most of the animals in this village were in one herd and had received 13 goats and one male goat from Harmanli. The situation was similar in Valche Pole where the percentage of infected goats was 25% (there was one infected male goat) and 17.5% in sheep (one infected ram). In Mramor the prevalence rate among goats was also high (20.5%). In the other areas most of the positive sheep and goats were purchased from Harmanli.

After discovering brucellosis, the National Veterinary Service took stringent measures according to EU legislation (Ordinance on the health require-

ments of sheep and goats in movement or transport between Bulgaria and the Member States of the EC, harmonized with Council Directive 91/68/EEC). By the end of 2008, 496 goats, 117 sheep and 7 cattle were destroyed in the affected villages. Animal owners were compensated according to the Law on Veterinary Activity.



Figure 1 – Geographical distribution of brucellosis in sheep and goats in Bulgaria for the period 7.VI. 2006 to 31.XII. 2008 Слика 1 – Географска дисшрибуција на бруцелозаша кај овци и кози во Бугарија во џериодош од 7.VI. 2006 до 31.XII. 2008

Brucellosis in humans

In 2005, a cluster of 37 cases of human brucellosis was registered, with 21 from the Sliven region (Table 2). Epidemiological investigations showed that, except for one person who worked in a sheep farm on Cyprus, all other patients worked and/or lived on animal farms in Greece. Nineteen workers, 14 of them from Sliven, were infected on one sheep farm located in Ekzohi, northern Greece. Data from National Veterinary Service for the same year showed that there were no Brucella positive sheep and goats in Bulgaria.

The first cases of brucellosis in sheep and goats in Bulgaria were discovered in 2006 at two villages in the Smolyan region. At that time, there were only 4 persons infected outside Bulgaria. The other 7 cases were considered as a cluster of autochthonous human brucellosis. Infected animals were detected on the farms of these persons.

Table 2 – Табела 2

Number of		Residence			
Year	infected persons (male/female)	District	No of cases	Probable country of infection / No of cases	
		Sliven	21		
2005	37	Yambol	8	Greece 36	
	(23/14)	Pazardjik	2	Cyprus 1	
		Plovdiv	6		
		Blagoevgrad	2		
		Plovdiv	2	Bulgaria 7	
2006	11	Smolyan	3	Greece 2	
	(6/5)	Yambol	2	Italy, Sicily 1	
		Varna	1	Tanzania 1	
		Kjustendil	1		
		Haskovo	53		
2007	58	Varna	2	Bulgaria 55	
	(31/25)	Sofia region	1	Greece 2	
	2 children	Blagoevgrad	1	Turkey 1	
		Plovdiv	1	-	
		Haskovo	6		
2008	14	Silistra	3	Bulgaria 9	
	(9/5)	Stara Zagora	3	Greece 5	
	` '	Targovishte	2		
Total	120	12	120	Bulgaria 71; abroad 49	

Cases of human brucellosis in Bulgaria, 2005–2008 Заболени лица од бруцелоза во Бугарија, 2005–2008

In 2007, a total of 58 cases of brucellosis in humans were identified. Fifty-four of them were connected with epizootic outbreaks in Harmanli, Valche pole and Mramor which took place at that time. These villages are situated in one region (Haskovo), bordering Greece and Turkey (Figure 2). All infected persons were owners of sheep and goats or had contact with these animals and/or consumed contaminated dairy products.

Because of measures taken and distribution of epidemiological information to the public in 2007, a significant reduction of the number of infected people was observed in 2008.

In 2005–08 there were a total of 120 infected people (69 men, 49 women, and 2 children), who were residents of 12 districts. Most of the cases were in the Haskovo region (59), followed by Sliven (21), Yambol (10), Plovdiv (9), Silistra (3), Smolyan (3), Stara Zagora (3), Blagoevgrad (3), Pazardjik (2), Targovishte (2), Varna (3), Kjustendil (1), and the Sofia region (1).



Figure 2 – Districts and villages in Haskovo region affected by brucellosis in sheep and goats Слика 2 – Обласии и села во регионош на Хасково зафашени со бруцелоза кај овцише и козише

Clinical signs in the acute phase of patients' illness included: fever, headache, anorexia, sweating, weakness, muscular and lumbar pain. Affection of various organs and systems was observed and manifested as: arthritis, epididymoorchitis, polyneuritis, skin rashes, hepato- and spleno-megaly, cystitis, endocarditis, myocarditis, pneumonia and uveitis [8]. *B. melitensis* biovar 3 was isolated from one person who worked in Greece and another who worked in Turkey. In all other cases the laboratory diagnosis was based on serological tests: Rose Bengal (Bul Bio – NCIPD), serum agglutination test (Bul Bio – NCIPD) and Brucellacapt (Vircell).

The results of the epidemiological investigations suggested that 45 persons (37.5%) were infected in northern Greece while working on sheep and goat farms. Four persons were considered as infected in Cyprus, Turkey, Italy and Tanzania, respectively. In both imported and autochthonous cases the mode of transmission was via contact with sheep and goats and/or consumption of milk and dairy products on Greek animal farms after working there for 3 to 36 months. In 14 cases the first clinical signs occurred during or shortly after the lamb/kid birth season of sheep and goats (December-May), in which contacts are the leading mode of transmission for the Brucella infection – contamination from vaginal discharges.

Discussion

The re-introduction of brucellosis in sheep and goats in Bulgaria is an important epizootiological and epidemiological problem for the society, health and veterinary services. Worldwide experience shows that brucellosis in sheep

and goats is among the most difficult diseases to eradicate [2]. The prevalence of infected sheep and goat herds in Greece was 5.13% tested in 2005 [6] and 0.23% in 2006 [7]. In 2006 only 1095 (5.0%) of the registered 21 969 herds of sheep and goats in Greece were tested [7], which is insignificant for a proper assessment of the epidemiological situation.

From 1998 to 2000, border formalities between Bulgaria and Greece were eliminated. There were numerous cases of movement of small ruminants and cattle to Bulgaria, especially during the summer months for grazing and watering. It is difficult to assess the volume of illegal movement of animals between the two countries which was the basis for the arrival of epizootic outbreaks and an outbreak in the population. As a result 496, goats 117 sheep and 7 cattle were destroyed, and 71 (59.1%) autochthonous human cases occurred. Cheap labour provided by Bulgarian citizens is attractive for the employment of workers in Greece, including animal holdings. This led to the infection of 45 persons from a total of 49 imported cases.

Our isolates belong to B. melitensis, biovar 3. According to the report of the Directorate General SANCO [2], this biovar is dominant in the Mediterranean region and rearing of infected sheep, goats and cattle. It means that the infectious agent wight be introduced to cattle which can also be infected with B. melitensis. This may explain the presence of 7 cattle in 3 villages with positive serological reactions. The question arises whether the infection of cattle is due to B. melitensis or B. abortus, because B. abortus is widespread in Greece (283 infected cattle herds in 2007). Goats are considered to be a major host of B. melitensis, biovar 3. In these animals after an abortion excretion of the infectious agent continues from 3 to 4 weeks [2]. Links between infections of humans and contact with small ruminants were established in Haskovo, Smolyan, Stara Zagora and Yambol districts. In the remaining regions with human cases, there were no infected animals. Our investigations found that the sale of infected goats from Harmanli without veterinary certificates in 11 villages of 3 districts was the most important means of dissemination of brucellosis in sheep and goats. Free movement of goods and of people between EU Member States is a fundamental policy of the Community but has some negative epizootological and epidemiological effects, one of which is the re-emergence of brucellosis caused by *B. melitensis* in Bulgaria. All Mediterranean countries which have problems with brucellosis in sheep and goats have similar problems with brucellosis in cattle caused by B. abortus. In 2005, Greece reported 283 infected herds -4.3% of those tested [6], and in 2006 -2.9% of those tested [7], and there is a risk of the spread of this type of brucellosis in Bulgaria. Animal owners, local authorities, processors and traders in animals and foods of animal origin must be informed about the necessary data for brucellosis and know the legal requirements for prophylaxis. As a supplement to the statutory requirements in the legislation, the NVS and the Ministry of Health have to implement additional and differentiated measures, especially in communities bordering endemic countries, with a view to early detection, containment and eradication of this dangerous zoonosis.

REFERENCES

1. Alton G. (1990): Brucella melitensis. In: Nielsen K., Duncan J., eds. Animal Brucellosis. CRC Press, Boston; 383–409.

2. European Commission. (2001): Scientific Animal Health Opinion – Brucellosis in sheep and goats. European Commission; DG SANCO, DOC-C.2/AH/R23/2001.

3. Likov B., Kamenov P., Boikovski I., Ralchev S., Tsankova S., Nenova-Poliakova R. (2009): Distribution of brucellosis in sheep and goats in the EU member states for the period 2005–2006 [in Bulgaian]. *Veterinarna sbirka*; 3–4: 19–21.

4. Semerdjiev B. (1951): Brucellosis. In: Semerdjiev B, ed. Sheep and goat diseases. [in Bulgaian]. Zemedelie; 106–19.

5. Russo G., Pasquali P., Nenova R., Alexandrov T., Ralchev S., Vullo V. *et al.* (2009): Reemergence of Human and Animal Brucellosis in Bulgaria. *J. Emerging Inf. Dis.*; 15: 34–8.

6. European Commission. (2006): The Community Summary Report on trends and sources of zoonoses, zoonotic agents, antimicrobial resistance and foodborne outbreaks in the EU in 2005. *The EFSA Journal*; 94: 3–288.

7. European Commission. (2007): The Community Summary Report on trends and sources of zoonoses, zoonotic agents, antimicrobial resistance and foodborne outbreaks in the EU in 2006. *The EFSA Journal*; 130: 3–252.

8. Cherveniakova T., Nenova-Poliakova R., Novkirishki V. (2008): Clinical features and therapeutic approach to the acute and chronic stages of epidemiologically connected brucellosis cases. [in Bulgarian]. *Contemporary Medicine*; 3: 19–24.

Резиме

ЕПИДЕМИОЛОШКИ КАРАКТЕРИСТИКИ НА БРУЦЕЛОЗАТА КАЈ ОВЦИ И КОЗИ ВО БУГАРИЈА: 2005–2008

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Цел: Да се анализира и презентира епидемиолошката и епизоотолошката ситуација на бруцелозата кај луѓето, овците и козите предизвикани од Brucella melitensis.

Мешоди: Анализирани се епидемиолошки извештаи, како и други официјални документи на ветеринарната и здравствената служба, во врска

со епидемиологијата и мерките за контрола на бруцелозата во Бугарија, 2005–2008 г.

Резулшаши: Во Бугарија инфекциите кај животни предизвикани од Brucella melitensis беа ерадицирани уште од 1941 година. Во 2005 година, неколку бугарски граѓани беа дијагностицирани со инфекција од Brucella во Референтната национална лабораторија. Сите случаи беа импортирани, најмногу од нив вработени на фарми со овци и кози во Грција. Во период од 4 години, бројот на инфицирани луѓе беше 120, дистрибуирани во 12 области. Епидемиолошките истражувања покажаа дека 45 (37,5%) беа инфицирани во Грција и 4 во Кипар, Турција, Италија и Танзанија. За сите други пациенти се сметаше дека се инфицирани во Бугарија. Тоа беа сопственици на инфицирани овци и кози или конзументи на контаминирани млечни производи со Brucella melitensis. Во јуни 2006 година, беа забележани првите епидемии со бруцелоза на фарми за овци и кози во областа на Смолен. До крајот на 2008 година, инфекцијата беше регистрирана во 16 села во 4 области. Просечната преваленција кај инфицираните стада беше 12,2% кај козите и 1,8% кај овците. За да се постигне ерадикација, 496 кози и 117 овци беа уништени.

Заклучок: Беше утврдено дека примарниот извор на инфекцијата е фармата во која нелегално беа внесени кози од Грција во 2005 год. Треба да се имаат предвид негативните епидемиолошки аспекти на слободното движење на луѓето и материјалните добра.

Клучни зборови: *Brucella melitensis*, бруцелоза кај луѓето, бруцелоза кај овци и кози, зооноза што се појавува.

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