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Gaps in Brucellosis Eradication Campaign in Sheep and Goats in Republic of Macedonia: Lessons Learned

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Aim To identify why "test and slaughter policy" for eradication of brucellosis did not significantly reduce the prevalence in sheep and goats in Macedonia.

Method Coverage of sampled vs expected number of sheep and goats, absolute number of positive animals, prevalence, frequency distribution, and classes of disease prevalence were retrospectively analyzed at the village level for 2004-2006. A comparative analysis of the disease prevalence in the investigated villages was also performed. The percentage of slaughtered animals was analyzed for 2000-2006.

Results We found differences between the expected and actual number of sampled animals, which were related to the type of livestock breeding. Traditionally maintained flocks and migratory flocks were considered to be responsible for the transmission of the disease. The absolute number of positive animals and the number of infected vs non-infected holdings did not decrease over the study period. Most of the villages had between 1 and 10 positive animals. Between 2000 and 2006, 55% of the positive animals were slaughtered, 41% in 2001 and up to 79% in 2002. Moreover, in 2005 and 2006, 34% and 53% of sheep and goats were found to be positive at the slaughter line, respectively, demonstrating that only 21%-23% of the infected animals were correctly removed from the herds.

Conclusion Based on the findings of this study, Macedonia changed its control strategy from "test and slaughter" to a vaccination policy for sheep and goats in 2008.

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Brucellosis in sheep and goats is a disease of major economic and zoonotic importance, and implementation of strategies for its control and eradication is essential in endemic areas (1,2). The aim of such strategies is to minimize the impact of the disease on human health, as well as on animal health and productivity.

The eradication or total elimination of brucellosis from a herd or flock, an area, or a country is a most often desired outcome, but one which is often very difficult to achieve (2,3). Different countries may require different strategies for the prevention and control of brucellosis in the population of small ruminants, depending on epidemiological and socioeconomic conditions. In deciding about a strategy, many factors must be considered, such as the type of animal husbandry, the geography of the area, the patterns of commerce, financial, technical and personnel resources available, the prevalence of disease, and compliance of the livestock owners (3-5).

Based on the prevalence of brucellosis in a country, its distribution, and the capacity of the veterinary services to control the epidemiological parameters, the EU Scientific Committee recommends three policies for control and eradication of the disease: 1) test and slaughter; 2) young animal vaccination with test and slaughter of infected animals; and 3) mass vaccination (6). The selection of the policy and the criteria for brucellosis-free status are clearly described by international organizations (7) and the appropriate EU directive (8). The "test and slaughter" policy was explicitly adopted in Macedonia in the middle of the 1970s. However, multiannual efforts based on the "test and slaughter" policy have not significantly decreased the prevalence. Many individual studies have been performed to determine the gaps in the system, but have not identified all factors responsible for the policy's failure. Thus, the Head Veterinary Office at the Ministry of Agriculture, Forestry, and Water Economy and with the support of the Faculty of Veterinary Medicine in Skopje launched an epidemiological study to elucidate the most important factors for the failure. It was designed to monitor the brucellosis infection for 2004, 2005, and the first half of 2006 and was the first attempt to follow up all critical points in the chain of brucellosis eradication campaign (9).

The aim of this study was to define the gaps in the current eradication system and to propose a redesigned strategy that included corrective measures essential for a successful eradication.

METHODS

During 2004, 2005, and 2006, a "test and slaughter" policy for the control of brucellosis in sheep and goats was implemented. According to the annual order, the designated veterinary practices were obliged to sample all animals in their appropriate epidemiological areas. These samples were tested using the Rose Bengal Test (RBT) (10) and positive flocks confirmed using enzyme-linked immunoassay (ELISA) in 2004 or the complement fixation test (CFT) in 2005 and 2006 (7,11-13). Positive animals were slaughtered in a sanitary slaughterhouse (6,8,14,15).

The epidemiological data for the present study were obtained from the two existing information systems in Macedonia, the National Epidemiological Information System and the Laboratory Information System, and were exported to Microsoft Access or Microsoft Excel for further analysis (16,17).

A census count of animals in each village was determined by the designated veterinary practitioners, who filled out and signed standardized forms. The total number of animals at the level of epidemiological unit or epidemiological area was calculated. For each epidemiological area, the proportion of sampled animals to total animals was calculated. This calculation was made to estimate the effectiveness of the designated veterinary practices at sampling all animals within their epidemiological areas. Fluctuation of animals at the village level was estimated by calculating the difference between the number of sampled animals from year to year. The prevalence at epidemiological area level was estimated as the number of positive animals in the epidemiological areas against the total number of sampled (tested) animals.

To determine the clusters of positive villages, the frequency distribution of the absolute numbers of positive animals within villages and the frequency distribution of the prevalence of the disease, also within villages, were used. Based on these results, villages were classified according to disease prevalence: 0.01%-1% for slightly affected, 1.01%-10% for medium affected, and 10.01%-100% for highly affected.

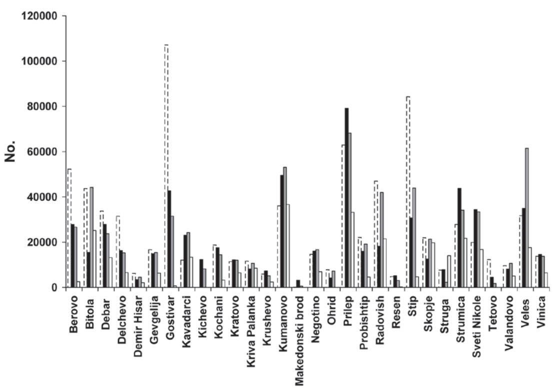
To check the consistency and effectiveness of the eradication strategy, the status of the villages (infected vs not infected) was monitored and compared from year to year.

Finally, the success in slaughtering of positive animals was calculated as the difference between the number of positive and slaughtered animals. For 2005 and 2006, the proportion of slaughtered animals was examined (retested) at the slaughter line, to estimate the accuracy in positive reactors identification.

RESULTS

The proportion of sampled animals out of the total (expected) census count of animals at the epidemiological area level is shown in Figure 1.

Figure 1.



Expected number of animals in each epidemiological area (dashed bars) and the absolute number of sampled animals during 2004 (closed bars), 2005 (grey bars), and the first half of 2006 (open bars).

TABLE 1. Absolute and cumulative percentages of disagreement (difference) between the number of sampled animals and the total census count of animals per village.

Difference	Absolute (%)	Cumulative (%)
±100	66	66
±200	12	78
±300	6	84
±400	3	88

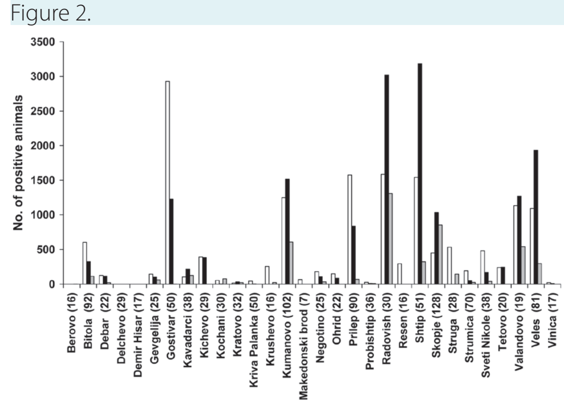
In most of epidemiological areas, there was a significant difference between the census count of animals and the number of sampled animals in 2004 and 2005 (Table 1).

The absolute number of positive animals at the epidemiological area level is shown in Figure 2 and prevalence is shown in Figure 3.

The prevalence did not uniformly decline across all epidemiological areas (Figure 2 and Figure 3). In addition, the epidemiological areas showed significant differences in the absolute number of positive animals (Figure 3). The prevalence decreased in some of the epidemiological areas, but the disease was not totally eradicated in any of them. Figure 4 shows the frequency of villages containing different numbers of positive animals. In the majority of positive villages, only 1-10 positive animals were found. Figure 5 and Table 2 show the frequency of villages that were categorized by disease prevalence.

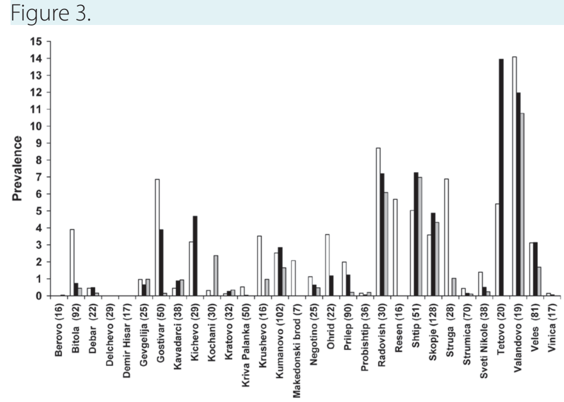
Among the villages that were negative during 2004, 68 (5.5%) became positive in 2005 and 48 (3.9%) in 2006. Additional 53 (4.3%) of the villages that were negative during 2005 became positive during 2006. Incomplete sampling during the study period was a significant problem. For example, 43 (3.5%) and 126 (10.3%) of the villages that were positive during 2004 were not examined at all during 2005 or 2006, respectively. Additional 82 (6.7%) villages that were positive during 2005 were not examined at all during 2006 (Table 3).

Figure 2.



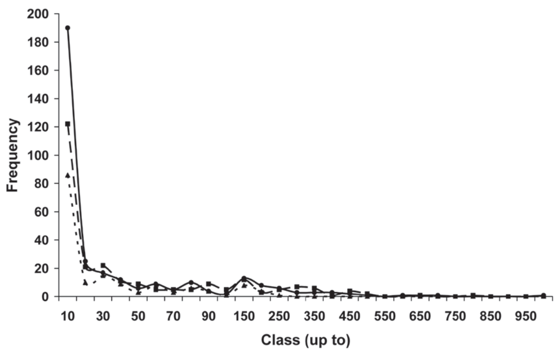
Absolute number of positive animals in 2004 (open bars), 2005 (closed bars), and the first half of 2006 (grey bars), according to the epidemiological areas.

Figure 3.



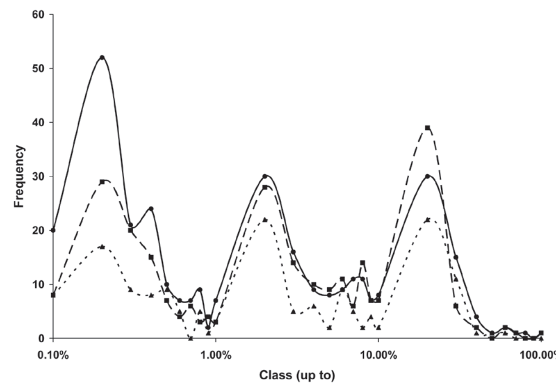
Prevalence of brucellosis in 2004 (open bars), 2005 (closed bars), and the first half of 2006 (grey bars) by epidemiological area.

Figure 4.



Distribution of the absolute number of positive animals in the villages. Full line shows the distribution in 2004, dashed line the distribution in 2005, and dotted line the distribution in the first half of 2006.

Figure 5.



Frequency of prevalence in infected villages. Full line indicates the frequency of prevalence for 2004, dashed line the frequency of prevalence for 2005, and dotted line the frequency of prevalence in the first half of 2006.

According to the annual order of the Head Veterinary Office from 2000 to 2006, all positive animals had to be slaughtered within one month of the determination of their status (Table 4). The local veterinary inspector receives the result directly from the laboratory and is obliged to organize the removal of animals in her or his designated region. The number of removed animals never reached 100%. Moreover, the number fluctuated greatly, from a minimum of 40.9% in 2001 to a maximum of 79.3% in 2002, with the average of 55.2% for all 7 years of observation.

TABLE 2. Number of villages within each of the three classes of prevalence

	No. of villages (% of total)		
Prevalence class (%)	2004	2005	2006
Low (0.01-1)	159 (49.2)	99 (38.7)	65 (41.1)
Medium (1.01-10)	109 (33.7)	106 (41.4)	57 (36.1)
High (10.01-100)	55 (17.0)	51 (19.9)	36 (22.8)
Total*	323 (100)	256 (100)	158 (100)

*The total number of villages sampled during the control and eradication campaign was different from year to year.

The number of positive animals at the slaughter line was far below the level of expectation (100%), reaching only 34% in 2005 and 52.8% in 2006 (Table 5).

DISCUSSION

Our study found many gaps in the chain of brucellosis eradication in Macedonia, which have contributed to the campaign's failure. The prerequisite for a successful "test and slaughter" policy is an exact estimation of the number of animals to be tested (18-20). Thus, veterinary practices are expected to sample all animals in their epidemiological areas. There was a significant disagreement between the number of expected animals and actually sampled animals, for which there are several possible reasons: 1) lack of field knowledge, leading personnel to over- and underestimate the number of sheep or goats; 2) lack of sufficient staff to fulfill the annual order, meaning that some veterinary practices are not able to sample all the animals in the

TABLE 3. Changes in the number of positive and negative animals in villages over the study period*

	2005 negative (%)	2005 positive (%)	2005 not examined (%)	2006 negative (%)	2006 positive (%)	2006 not examined (%)
2004 not examined	184 (15.0)	49 (4.0)	26 (2.1)	93 (7.6)	21 (1.7)	145 (11.8)
2004 negative	485 (39.6)	68 (5.5)†	91 (7.4)	303 (24.7)	48 (3.9)†	293 (23.9)
2004 positive	141 (11.5)	139 (11.3)	43 (3.5)‡	108 (8.8)	89 (7.3)	126 (10.3)‡
2005 not examined				41 (3.3)	11 (0.9)	108 (8.8)
2005 negative				383 (31.2)	53 (4.3)†	374 (30.5)
2005 positive				80 (6.5)	94 (7.7)	82 (6.7)‡

*The percentages given in parentheses were calculated according to the total number of villages examined (n = 1.226) during all 3 y of observation.
†Villages that converted from negative to positive status in the second year of the study.
‡Villages that were positive in the first year of study, but were not examined at all in the second year of the study.

TABLE 4. Removal rate of positive animals between 2000 and 2006

Year	No. of tests	No. of positive tests (% prevalence)	No. of animals removed (% of total removed)
2000	670 919	2026 (0.3)	1115 (55.0)
2001	752 397	4059 (0.5)	1660 (40.9)
2002	806 861	10 102 (1.3)	8014 (79.3)
2003	804 067	7029 (0.9)	3613 (51.4)
2004	610 957	15 494 (2.5)	8448 (54.5)
2005	667 714	15 872 (2.4)	9750 (61.4)
2006	298 951	4701 (1.6)	2055 (43.7)
Average (all years)	658 838	8469 (1.4)	4951 (55.2)

TABLE 5. Results of retesting a proportion of positive animals at the slaughter line

Year	No. of animals slaughtered	No. of retested animals according to their status at the slaughter line	
		positive (%)	negative (%)
2005	1374	604 (44.0)	770 (56.0)
2006	2036	1086 (53.3)	950 (46.7)

field; and 3) over-sampling, when more than one sample is taken from one animal. These reasons should be considered when evaluating any program for disease control, not only in Macedonia (5).

To correct these errors, improved and strict criteria for designating veterinary practices, as well as implementing permanent animal identification and registration systems, are needed. This will enable precise calculation of the number of animals at the level of individual farms or holdings. This is already in progress in Macedonia, as a project managed by the European Agency for Reconstruction and implemented by the Head Veterinary Office of Macedonia. Such identification and registration systems would allow flocks to remain under strict surveillance and all animal movements to be monitored. In parallel, an efficient and well organized veterinary service for surveillance should be put in place (2,6).

Fluctuation in the number of sampled animals in the villages raises questions about the capacity of the whole system to control animal movement and about the success of sampling in the villages. This fluctuation reflects incorrect practices and underscores the fact that no system exists to ascertain the real number of animals in the villages. Macedonia plans to implement an Animal Identification and Registration System and a Veterinary Information System, which should improve the situation.

Our study showed that the "test and slaughter" system was not successful in eradicating disease in most of the regions of Macedonia. Most of the villages had only 1-10 positive animals and, theoretically, it should be easy to remove all positive animals from such villages. However, the prevalence did not decrease from year to year, but rather for every year of the study, there were clusters of villages with low, middle, and high prevalence. This leads to the conclusion that although the control measures were applied each year, a reduction in the number of positive animals did not occur.

The evaluation of the disease status in the villages showed two important trends. There was a significant number of villages that were negative in the first year of the study but became positive in the second year, reflecting the lack of consistent control measures. There was also a significant number of villages that were positive in the first year of the study and were not examined at all in the following years, indicating a lack of priority planning by veterinary practices. On the other hand, a significant number of villages converted from positive to negative, which shows that consistent measures lead to visible results.

One of the most important factors responsible for the eradication campaign's lack of success was probably the failure to remove positive animals. The percentage of positive animals removed ranged from 40.9% to 79.3%, with an average of 55%. This was a consequence of frequent funding problems because of which farmers could not be reimbursed for animal removal. This confirms that necessary financial resources are a critical prerequisite for any successful eradication campaign (4).

Even more discouraging are the results of animal retesting at the slaughter line, which is a good way to monitor the success of correct identification during the removal process. Only 21% (2005) and 23% (2006) of the total number of positive animals were correctly removed. There can be several reasons for incorrect removal of animals, including the lack of official control during identification of positive animals (not enough official veterinarians employed), lack of supervision by the respective veterinary practices, exchange of the temporary ear tags by the farmers (slaughtering of invaluable animals), and lack of consequences for noncompliance with official measures.

As a result of this study, the control strategy of Macedonia was changed in 2008, from the "test and slaughter" approach to vaccination for sheep and goats with Rev 1 vaccine applied intraocularly (21-24). Although there are no precise recommendations for all conditions and countries, it is important to define areas with high, intermediate, and low prevalence of the disease (6). Consequently, the country was divided into three vaccination regions: non-vaccination in regions traditionally free of brucellosis for more than 20 years; vaccination of replacement animals in low-prevalence regions; and full vaccination in regions of high prevalence.

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