

INSIGHTS INTO THE PROBLEM OF *B. MELITENSIS* AND RATIONALIZING A VACCINATION PROGRAMME IN ISRAEL

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Abstract: *Aim:* To present the problem of brucellosis caused by *B. melitensis* in Israel and to develop a rationalized control programme and eradication campaign in small ruminants.

Methods: CFT, an OIE prescribed test and a confirmatory method, was used as a confirmatory test in support of legislation to compensate the farmers following a stamping-out policy. Positive reactors were cultured for *Brucella* spp. in order to establish epidemiological data. Vaccination using Rev. 1 Elberg vaccine strain, passage 101, 1970, has been implemented as an ocular method since November 1997.

Results: Brucellosis due to *B. abortus* in cattle has been eradicated in Israel in beef cattle and in dairy cattle since 1984 and 1985, respectively. *B. melitensis* has emerged in small ruminants since 1970 and become endemic in the country. An eradication campaign has been carried out since 1993, as an interim programme between 1993 to 1995 and as a full programme up to 1997. The vaccination of pregnant animals has led to abortions and, as a result, this policy was abandoned. To date, only young replacement females are vaccinated.

Conclusion: Following a national eradication campaign the number of human cases declined significantly but ceasing the campaign has led to re-emergence of the disease. Cattle brucellosis due to *B. melitensis* has been successfully controlled with limited sporadic emerging events that have occurred since this campaign. A test and slaughter programme that is integrated with due vaccination of young replacement animals is proposed to facilitate control of the disease with the intention of achieving complete eradication.

Key words: *B. melitensis*, control, vaccine, serology, bacteriological cultures.

Introduction

Israel has eradicated *B. abortus* since 1984 in dairy cattle and since 1985 in beef cattle, respectively [1]. The successful eradication campaign was achieved following application of the test and slaughter programme simultaneously with the use of a single dose vaccination of female calves by *B. abortus* vaccine strain S19. The S19 is a live attenuated vaccine strain, developed by Buck back in the 1930s [2] and since used worldwide with significant success [3]. Interestingly, because S19 resembles smooth field strains due to sharing identical smooth O-antigen structure, the application of the S19 vaccination programme in the herds mostly resulted in eliciting positive serological responses to *Brucella* smooth antigen [4] specifically if a full dose had been used by a sub-cutaneous application. Moreover, because strain 19 is a live attenuated vaccine, its application in pregnant cows would have caused abortions. Despite these inconvenient adverse effects, a test and slaughter programme could be carried out successfully indicating that S19 is a safe vaccine for cattle and would not cause significant problems in integrated vaccination and tests and slaughter programmes.

This information was known in the 1970s and 1980s. However, new serological tests were sought based on ELISA that were reported to have improved sensitivity and specificity as well as allowing to distinguish between vaccination and infection humoral antibodies [5, 6]. At this time, Israel has chosen CFT for the implementation of the serological tests using standardized reagents according to the 2nd international standard anti-*Brucella abortus* serum [7]. Secondly, it was fully acknowledged that reducing the vaccine dose, or changing the route of application to a conjunctival inoculation could prove safe in both reducing the humoral response and preventing abortions in pregnant animals. Several papers were then published on the new developments and their possible endorsement in endemic areas where vaccination was part of a control or an eradication campaign [8]. The promise made by these improvements that vaccination would not hamper serological surveillance has further led scientists to propose adult vaccination in endemic areas as a possible means of increasing vaccination coverage to the herds [9] and simultaneously, of providing a better solution to developing countries suffering from a high incidence of human cases that could not afford animal depopulation due to budget concerns.

By improving the serological tests as well as better understanding the adverse effects associated with the application of the vaccine in pregnant cattle while preventing abortions in the herds, most western countries including Israel have achieved complete control of the disease and have subsequently completed the eradication programme.

With the opening of the Israeli borders to the West Bank a slow but an ongoing process of change towards involving small ruminant industry as a significant reproduction system has become prominent. Israel has gained a reputation in its dairy herd management and reproduction system that yielded increasing milk levels to record figures in the world. With the advent of the small ruminant industry as a significant part of the Israeli dairy system and combined with the Bedouin tradition of nomadic way of living, *B. melitensis* was eventually introduced into the country. This has been recognized immediately by a slow but steady increase in the incidences of human brucellosis. As this specific disease has remained underreported, it is assumed that the true figures have by far exceeded the reported numbers.

This was the beginning of a new era of brucellosis in Israel limited to *B. melitensis* only, as a single species that causes the disease. A major development in the field of vaccination had been achieved in parallel by Dr. Elberg when developing in the mid 1950s a *B. melitensis* vaccine endowed with equal, and presumably improved protection not only against *B. melitensis* in sheep and goats, but further extended to be a better vaccine also against cattle brucellosis. However, the vaccine has shown similar adverse effects to those shown in the past to occur with the *B. abortus* S19 vaccine. When applying *B. melitensis* Rev. 1 vaccine in adult ewes and goats the vaccine induced abortions in pregnant animals and persistent humoral responses developed that hampered serological surveillance programmes due to cross reacting with anti-smooth antibodies that are common between the vaccine strain and the field cases. Further, despite the homogeneity of the *Brucella* genus regarding its capacity to cause abortions, collected data has shown that the disease in sheep and goats could involve other complications not appreciated beforehand. One important factor was the invalidity of the application of the known bovine serological tests to small ruminants regarding their performance in diagnosing *B. melitensis* infections in small ruminants in comparison to *B. abortus* in cattle. Moreover, the disease amongst humans presents complications that were not duly addressed by the standard diagnostic approaches developed for *B. abortus* in cattle, requiring that the classical bacteriological culturing of the strain be implemented as an important flowchart for confirming human cases. Nowadays, new molecular tools have been added aimed at improving human and animal diagnosis as well as showing promise in increasing our understanding of the pathogenesis of the disease [10–12].

Our findings in Israel, and similar observations reported in France, have added new insights to our understanding of *B. melitensis* infections in dairy cattle. It was always almost an axiom that a significant profile of the pathogenesis of the disease is the intimate linkage between the *Brucella* species association with their natural hosts and the induction of abortion storms in the herds following infection of pregnant animals. With the advent of the dairy cattle

diagnosis, it was then found by the Israeli team that the milk ring test (MRT) test, the specific tool that was by consensus accepted as an important tool in monitoring dairy herds for brucellosis, failed in identifying an infection in one of the larger Israeli dairy herds that encountered *B. melitensis* biovar 1 infection. Until the bulk milk MRT eventually turned positive, two important events happened. Firstly, a significant number of herdsmen contracted brucellosis but unfortunately they were misdiagnosed as negative by the serological tests at the beginning of the event, leading to postponing their treatment. Secondly, when the herd was eventually diagnosed positive by serology and by bacteriological cultures, disease prevalence in the herd had reached about 60% of the milking cows. It was then understood that due to this heavy rate of infection, the complete destruction of the whole herd was a necessary step in order to stop this problem. Despite taking precautionary safety measures in the slaughterhouse, additional human infection of the slaughterhouse personnel occurred indicating the significance of the *B. melitensis* health risk. An important lesson of this case was a decision taken that animal slaughter should be carried out only after drying the infected cow in order to prevent exposure of the slaughterhouse personnel to infected aerosols [13]. In addition, the veterinary services issued a regulation that in order to minimize human risks of infections *Brucella* culture positive animals be euthanized and meat would not be allowed for consumption.

The failure by MRT to diagnose the disease in real time has been recently readdressed in our laboratory. Interestingly, we observed a delay in the humoral response of the cows until after parturition that was simultaneously accompanied by *Brucella* secretion in the milk. It is still a premature observation that does not allow us to conclude whether improved milk i-ELISA tests could have overcome the problem.

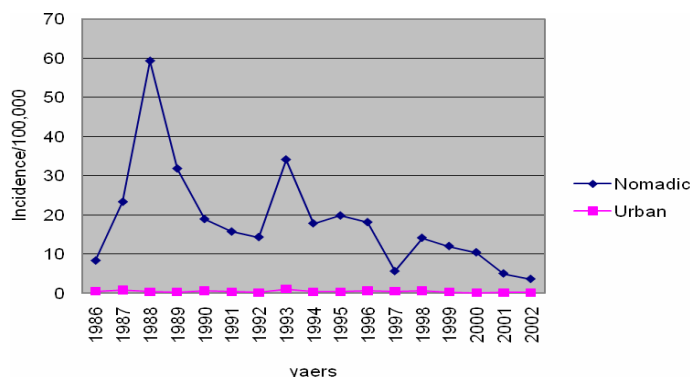
The Israeli national control programme

Because *B. melitensis* was endemic in Israel, and following dairy herd infections that ensued [14], the veterinary services have developed a two-step eradication campaign, addressing first heavily infected foci throughout an interim campaign followed by a full coverage of all the flocks in Israel by conducting a test and slaughter programme of the whole adult population by CFT. The decision on the CFT application was taken due to the test being an OIE prescribed method as well as being considered a confirmatory method for the purpose of a test and slaughter programme against compensation due to its excellent performance as judged by its high sensitivity and specificity. Moreover, this test was in use by the Israeli laboratory following calibration against the international serum standard that also afforded distinguishing true positive reactors from Rev. 1 vaccination antibody response. Within this context, it was accep-

ted also that a vaccination policy would be based on a single administration of the Rev. 1 vaccine to all ewe-lambs and female kid-goats between 2 to 6 months of age. This policy was aimed at establishing immune protection of the flocks without hampering the serological surveillance.

The whole programme was pursued between 1993 and the beginning of 1997. The interim programme had been carried out from 1993 to 1995 and successively continued to a national eradication campaign from 1995 to 1997. During the interim programme, animal prevalence was above 6% and by the test and slaughter activity it was reduced to about 3% in the following years. Throughout the campaign, in the interim programme 1635 flocks (59,901 ewes and goats) and in the eradication campaign 4,292 flocks (195,176 ewes and goats) were tested. More than 40,000 ewes and goats were slaughtered establishing around 72% of the flocks clear of the disease [14].

Interesting data have been obtained regarding human brucellosis. It was expected that human brucellosis would drop sharply simultaneously with the reduction of disease prevalence in the small ruminant population. Figure 1 shows that this expectation was not achieved and human brucellosis continued to pose a significant concern during the campaign. In addition, the Ministry of Finance called off the campaign in 1997, due to lack of financial resources available for a continued slaughter against compensation.



Source: Data retrieved from the Department of Epidemiology, Ministry of Health, Israel.

Figure 1 – Human *B. melitensis* cases in Israel in the years 1986–2002 (incidence per 100,000)

Слика 1 – Заболени луѓе од *B. melitensis* во Израел во годиниие од 1986–2002 (инциденца на 100.000)

Importantly, figures on the incidence rate of human brucellosis started to drop sharply immediately after showing record low numbers of new cases in the years 2000 to 2002. Unfortunately, because of ceasing the brucellosis cam-

paign, animal brucellosis in sheep and goats has re-emerged, leading to the development of new human cases in numbers reaching those of the years that preceded the campaign, i.e. figuring around 120 culturally confirmed cases annually. This unfortunate consequence is a reminder of the human risks encountered due to small ruminant brucellosis by *B. melitensis*. Figure 2 depicts data relevant to the human cases identified by strain isolation from 2002 to 2008, showing that a steady increase in numbers occurred. As *Brucella* isolation is considered a gold standard test, this number is an under-report of the true number of human cases that may have been observed during a serological survey.

Human cases, 2002–2008

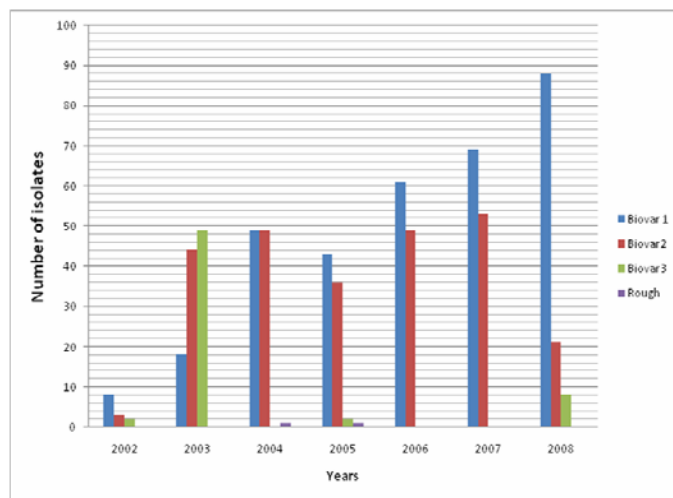


Figure 2 – *B. melitensis* human isolates (distribution according to biovars) received in the National Brucellosis Reference Laboratory in the years 2002 to 2008
Слика 2 – *B. melitensis* човечки изолати (дистрибуција според соеви) примени во Националната референсна лабораторија во годините од 2002 до 2008

One of the major findings during the eradication campaign was that Rev. 1 showed adverse effects that were encountered in large numbers. Israel is one of the few countries that have reported on a human Rev.1 infection that did not involve occupational disease. Further, our field results have proven horizontal transfer of Rev. 1 to non-vaccinated ewes that also included rough morphogenesis of the strain. We could also show that flocks vaccinated with the unqualified vaccine did not elicit strong flock protection and many vaccinated animals have shed a field strain in their milk [14]. All of the accumulating data have led us to hypothesize that the quality of the vaccine was not maintained during its

preparation by several commercial manufacturers. Dr. Banai was fortunate in establishing a direct correspondence with Dr. Elberg, developer of the Rev. 1 strain [10], resulting in the understanding that an original seed stock of the vaccine strain had been kept lyophilized in Dr. Elberg's laboratory. The passage 101, 1970 was assigned to the true Elberg strain as confirmed and deposited in European Pharmacopeia as the vaccine seed stock that best resembles the characteristics of the original strain. In the past, Israel participated in the pilot field tests that have shown the safety of the vaccine in ewe-lambs by confirming lack of horizontal transmission of the strain even after several passages in pregnant ewes [15, 16].

Having this information, Israel identified a manufacturer that confirmed his seed stock as originating in the European Pharmacopeia deposit. This vaccine was made available for the conjunctival inoculation method with minimized dose number to 5×10^8 cfu. The assumption that conjunctival vaccination would be a safer approach also for pregnant animals was noted from the literature [9], leading in Israel to instigating a mass vaccination campaign with the new vaccine that included intensively managed flocks. Despite this literature, because the vaccine was used also in pregnant ewes, abortion storms due to the vaccine ensued and further application of this vaccine in adult animals has been totally banned since 1999. Replacement animal vaccination of only female livestock at age between 2 to 6 months was then enforced, using a specific ear tag to indicate that the animal had been duly vaccinated according to the new regulations.

Table 1 shows that during the beginning of the new campaign, solely based on Rev. 1 vaccination restricted to the young progeny, mixed flocks existed that simultaneously included vaccinated ewes and goats, as well as unvaccinated ones (or animals that had been vaccinated by other commercial vaccines not confirmed to be originating from the Elberg 101 strain). It can be seen that serological brucellosis shown in the vaccinated group was slightly reduced compared to the unvaccinated group in the same flock, suggesting that the vaccination campaign had helped in protecting the herds. This, however, did not correlate with the bacteriological culturing results that have proved ewes still secreting a field strain in their milk. It could thus be suspected that the young progeny was most likely suckling milk contaminated by *B. melitensis* field strain from both vaccinated and unvaccinated ewes, perpetuating the disease to the next generation. Moreover, we have realized that, despite organization and regulations, the veterinary services failed to vaccinate all the young replacement animals in the time period that was safe for young replacement animal vaccination, leading again to the development of unprotected animals in nomadic flocks. It could then be argued that these unvaccinated animals were the source of spreading the disease among the Israeli small ruminants.

Table 1 – Табела 1

Percentages of serologically positive B. melitensis cases in flocks with a mixed Rev. 1 vaccinated and unvaccinated animal population
Процентни на серолошки позитивни B. melitensis случаи
во стада со мешана Rev. 1 вакцинирани и невакцинирани популации
на животињи

Place	Flock	Isolate	Vaccinated		Unvaccinated	
			Total No. animals	% positive (No)*	Total No. animals	% positive (No)*
Ku.Mu	Sheep	Human, b2	14	42.8% (6)	41	60.9% (25)
Shu.Yu	Sheep	Un-vacc sheep, b3	51	17.6% (9)	44	29.5% (13)
Mu.Sam	Mixed (s+g)	Un-vacc Vaccinated sheep, b2	60	33.3% (20)	49	69.3% (34)
Ab.Sud	Sheep	Un-vacc Vaccinated sheep, b2	16	25.0% (4)	18	61.1% (11)
M.S.M.	Sheep	Un-vacc sheep, b2	4	25.0% (1)	10	60.0% (6)
Di.Mu	Sheep	Un-vacc Vaccinated sheep, b2	5	40.0 (2)	25	8.0% (2)
A.S.M.S.	Sheep	Un-vacc sheep, b2	22	50.0% (11)	282	31.9% (90)
A.S.T.A.	Mixed (s+g)	None	54	25.9% (14)	122	49.1% (60)
A.G.A.S.	Sheep	None	14	7.1% (1)	69	44.9% (31)
Total			240	29.6%	660	46.0%

*The number of animals tested positive by the serological method are indicated in parentheses.

Table 2 shows that unvaccinated animals as a source of spreading the disease was only a partial explanation of the spread of the disease amongst the flocks. Inasmuch as intensively managed flocks, in which vaccine coverage was duly covering 100% of the animals or close to 100%, also contracted *B. melitensis*, with animal prevalence reaching similar infection rates to those seen in un-vaccinated animals. In such highly protected flocks it was not fully understood how *Brucella* was transmitted into the farm and why such a large number of animals contracted the disease despite being duly vaccinated. Moreover, at least some of the infected ewes were in close proximity to vaccination and yet *B. melitensis* was isolated from these animals.

Table 2 – Табела 2

B. melitensis infections in three intensively managed flocks that have been duly vaccinated with ocular Rev.1 Elberg vaccine strain
B. melitensis инфекцији во три интензивно контролирани стада кои биле навремено вакцинирани со Rev.1 Elberg vaccine strain

Farm	Herd size	Total No. positive by serology	<i>B. melitensis</i>
T. E.	358 sheep	45 (12.5%)	Biovar 1, atypical (Banai M, 2002)
K. U.	178 sheep	78 (43.8%)	Biovar 1, susceptible to penicillin
A	96 goats	11 (11.4%)	Biovar 1

A similar situation was observed amongst our dairy cattle that were duly vaccinated with S19. Here, the quality of the S19 vaccine had never been doubted and this vaccine had been duly applied as a full, subcutaneous dose to our dairy and beef cows. Vaccinated calves in close proximity to their vaccination contracted *B. melitensis* at a similar rate to adult animals that were remote from their vaccination time. Because *B. melitensis* does not normally elicit abortions in cows, the infected cows usually did not abort. However, in at least one of our dairy farms *B. melitensis* biovar 1 infection has led to abortions [14].

In conclusion, our data support previous publications regarding vaccination programmes. In a single abortion about 10^{13} organisms are identified in one gram of placenta. This huge number of organisms poses a risk of spreading the disease in the farm. The fact is that vaccination is basically intended to minimize abortion cases and thus prevent mass contamination of the farm with subsequent infection of the population. The vaccine is less significant in protecting the population from contracting the disease.

Since 1994, dairy cattle infection that was common in the 1990s was almost sporadic at the end of the century. There was only a single incident of dairy cattle brucellosis in a big dairy herd that very likely resembled previous reports in the 1990s. In this case, the first information on the infection stemmed from a single cow that gave birth to a premature calf and was tested positive by the serological tests. The herd was then tested immediately by MRT identifying several cows that were positive. Bacteriological culturing of milk samples then confirmed the infection in these cows that was caused by *B. melitensis* biovar 1. In a different part of the area (therefore not expected to be epidemiologically linked to the first event) additional farms contracted the disease. Despite these two reports, cattle brucellosis has significantly dropped since 2000, possibly

due to changes that ensued in the dairy cattle industry during this time. Most dairy farms have joined a revolutionized change in farm management, merging small units into large ones and significantly increasing bio-security measures on the farms. Thus, awareness seems to be a key factor in preventing disease from being introduced into the farm. We have thus concluded that combining vaccination with bio-security measures is expected to build up an effective barrier that prevents the disease from being introduced into the farm.

Conclusions

Based on the literature review and the experiences gained in Israel, the following conclusions might be formulated:

- *Brucella* species are strongly associated with specific natural hosts, inasmuch as to justify their taxonomical separation into nomen-species according to this criterion [17];
- Because most *Brucella* species are zoonotic, human brucellosis emerges as a public health problem in endemic regions. *Brucella melitensis* is the causative agent of small ruminant brucellosis and is the most severe disease for humans, causing Malta fever;
- Israel has eradicated *B. abortus* from dairy cattle since 1985 and no further cases have been identified since then. *B. abortus* vaccine S19 is applied in female calves aged between 3 to 7 months;
- *B. melitensis* emerged in humans in the 1970s, and the disease rapidly persisted in the population. Sheep and goats were the source of the disease in humans;
- By close contact with infected small ruminants, despite S19 vaccination, at the beginning of the 1990s dairy cattle contracted *B. melitensis* in several large farms, and some beef cattle. Humans were infected due to dairy cattle *B. melitensis* infections;
- Israel instigated a control programme divided into an interim period from 1993 to 1995 and successively continued with a full national programme until 1997. The programme was carried out based on a test and slaughter policy using CFT as a confirmatory method. Bacteriological cultures were used to confirm flock infection and provided presumptive typing of the causative agent;
- Throughout the programme, more than 40,000 sheep and goats were slaughtered. The financial burden of this programme that amounted to some millions of Israeli Shekels, eventually led to termination of the programme. Instead, Rev. 1 vaccination was enforced, using the assign-

ned Elberg strain passage 101, 1970 vaccine and restricting the vaccination to only young replacement females aged between 2 and 6 months;

- As a result of the campaign, human brucellosis dropped to record low numbers. Human brucellosis had been slowly and steadily increasing since 2002 because of re-emergence of the disease in sheep and goats flocks following termination of the national eradication campaign and despite adhering to an ongoing national vaccination campaign;
- For unexplained reasons, the increase in small ruminant brucellosis did not involve transmission of the disease to dairy cattle, as was the case in the 1990s. It is assumed that enforcing bio-security has added a significant impact to this success.

Recommendations

This report shows promise in achieving, in the near future, successful control of the disease in Israel. This can be achieved by combining regulatory activities with monitoring and vaccination, as illustrated below:

- Regulatory activities should include control of animal movement by registration of farms and ear tagging of animals;
- The state should support animal trade and sales in the market by certifying brucellosis-free farms. Replacement livestock in places that are undergoing an eradication programme might be obtained from such farms;
- Livestock aimed at the market should be monitored by serological tests prior to moving the animals from the farms;
- Intensively managed farms should be monitored annually by milk culture of the bulk tank and serological testing of the males (because in Israel males are not vaccinated they could be used as a sentinel);
- Human brucellosis should indicate a possible infection in a farm. The veterinary services should conduct an epidemiological examination to find the source of infection. The farm should then be eradicated by a test and slaughter programme until declared free of the disease;
- Vaccination should be restricted to the female ewe-lambs and kid-goats aged between 3 and 6 months in order to confer protection on the livestock while simultaneously minimizing vaccine strain cross reactive antibodies that may hamper serological diagnosis. Elberg strain 101, 1970, should be used as the official vaccine in Israel.

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Резиме

ОСВРТ НА ПРОБЛЕМОТ СО *B. MELITENSIS* И РАЦИОНАЛИЗАЦИЈА НА ПРОГРАМАТА ЗА ВАКЦИНАЦИЈА ВО ИЗРАЕЛ

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Цел: Да се прикаже проблемот со бруцелозата предизвикана од *B. melitensis* во Израел и да се развие рационална програма за контрола и кампања за ерадикација на болеста кај малите преживари.

Методи: CFT, препорачан тест од ОИЕ и метод за потврдување, користен е како метод за потврдување во поддршка на легислативата за финансиска компензација на фармерите согласно политиката на отстранување на заразениот добиток. На оние животни кои позитивно реагираа им беа правени култури за *Brucella spp* за да се добијат епидемиолошки податоци. Вакцинација со Rev. 1 Elberg вакциналниот вид, пасажа 101, 1970, беше имплементирана како окуларен/конјуктивален метод од ноември 1997 година.

Резултати: Бруцелозата предизвикана од *B. abortus* кај говедата во Израел е ерадицирана помеѓу стоката за производство на месо од 1984 година, а кај млечните крави од 1985 година. *B. melitensis* се појави кај ситните преживари во 1970 година и прерасна во ендемија на територијата на целата земја. Во 1993 година се започна со кампања за ерадикација, во интервалот од 1993–1995 година се спроведуваше како привремена програма и како целосна програма до 1997 година. Вакцинацијата на бремени животни предизвикуваше абортуси на плодовите, па овој пристап беше напуштен. Денес, се вакцинираат само млади женки за замена како подмладок.

Заклучок: По преземањето на националната кампања за ерадикација на бруцелозата, бројот на заболени луѓе од бруцелоза значително се намали, но прекинот на кампањата доведе до повторна појава на болеста. Бруцелозата кај говедата предизвикана од *B. melitensis* успешно беше контролирана, а само повремено се појавуваа спорадични инциденти. Програмата за тестирање и уништување на животните заедно со вакцинирањето на

младите животни се предлага за поуспешна контрола на болеста и тенденција за постигнување на комплетна ерадикација.

Клучни зборови: *B. melitensis*, контрола, вакцина, серологија, бактериолошки култури

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