

RESEARCH ARTICLE

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Control of brucellosis in cattle from Durres and Lushnja complexes through the application of *Brucella abortus* rb51 vaccine control of brucellosis in cattle

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Abstract

Brucellosis is one of the most important zoonotic diseases, epidemiological and bacteriological data of Food Safety and Veterinary Institute in Tirana, confirms the presence of this infection in all farm animals and is extended in the whole country. Monitoring of the epidemiological situation through serological tests carried out in cattle complexes in Durres and Lushnja, confirmed that the prevalence of brucellosis in these complexes were respectively 38.4 and 21.8%. Given of a considerable number of abortions, number of pathological specimens for the isolation of the cause of abortions, were from aborted fetuses, parenchymatous organs of the lymph glands of aborted cows and sera-positive results with serological tests. Based on morphological, biochemical characteristics and agglutination test with typo specific sera causes of abortions were identified as *Brucella abortus* biotypes 6 and *Brucella melitensis* biotypes 3, results confirmed by the reference laboratory VLA-London. In this context, the application of the control / eradication strategy of this infection in cattle from Durres and Lushnja complexes through the use of vaccine with strain *Brucella abortus* RB51 and evaluation of vaccine immunogenicity through brucelina PPD allergen manufactured by us is the main objective of this study. Through the use of this vaccine defined by this study, we obtained the control of *Brucella* infection in both complexes included in the study and 24 months after the application of the vaccines, complexes are considered to be "free" from brucellosis.

Key words: *Brucella abortus* RB51, serologic tests, cattle complexes, vaccination.

1. Introduction

Brucellosis is a zoonotic disease with great social and economic impact in our country and in many other developing countries. Presence of *Brucella* infection in small ruminants, mixed types of breeding in farm animal, uncontrolled emigration of animals, could be among the causes of cattle infection.

In this situation, in the complex of fighting measures of brucellosis, main importance is the diagnosis in time of this disease and the application of control/eradication strategies.

Therefore, knowledge of the prevalence and incidence of this infection, isolation and identification of the cause of this infection in farm animals, evaluation of serological and allergic tests and development of control / eradication strategies of this infection, continue to be a permanent object of science and veterinary practice.

2. Materials and Methods

2.1 Monitoring of the epidemiological situation of brucellosis in cattle complexes.

Study on monitoring of the epizootic situation of brucellosis in cattle in Durres and Lushnja complexes

is extended in the period of time 2010-2011. Given of a considerable number of abortions, 89 abortions in Durres complex and 7 abortions in Lushnja complex, monitoring of the epizootic situation of brucellosis was performed by serological tests. Serums resulting suspicious and positive were retested with iELISA test.

2.2 Isolation, identification and characterization of *Brucella* spp. in aborted cattle.

Given of a considerable number of abortions, number of pathological specimens for the isolation of the cause of abortions, were from aborted fetuses and from sera-positive cows resulted with serological tests.

2.3 Brucellosis control through vaccination and immunization schemes.

For immunization of cattle, we used *Brucella abortus* RB 51 vaccine, approved by the European Commission [4].

The vaccine was applied when the two complexes of cattle were considered virtually "free" by brucellosis. Before and after application of the vaccine and at intervals in every 6 months for a period

3. Results and Discussion

of 24 months are carried out tests in the absence or presence of anti-LPS antibodies with iELISA and RB test.

In vaccinated animals, chosen at random, 1-2 months after the application of vaccination schemes, were evaluated immunogenicity skills of the vaccines through allergic immune response.

3.1 Monitoring of the epidemiological situation of brucellosis in cattle complexes.

Serological examinations carried out on cattle from Durres and Lushnja complexes, confirmed the high prevalence of *Brucella* infection, that was respectively 38.4% and 21.8% of the total cattle tested.

Table 1: Serological control of cattle with Rosa-Bengal, RA and iELISA-blood serum serological tests in Durres and Lushnja complexes.

Date of analysis	No of samples tested	Positive serums analyzed with serological tests.			Eliminated cattle	% of positivity of cattle
		RB	RA	iELISA		
Testing I	359	106	93	110	110	
Testing II	249	25	16	24	24	
Testing III	221	4	2	4	4	
Testing IV	217	-	-	-	-	38,4%
Testing V	436	-	-	-	-	
Testing VI	668	-	-	-	-	
Total -Durrës	2244	131	111	138	138	
Testing I	312	22	20	25	25	
Testing II	287	23	20	26	26	
Testing III	261	4	3	4	4	
Testing IV	256	10	9	10	10	21,8%
Testing V	246	5	2	3	3	
Testing VI	256	-	-	-	-	
Testing VII	406					
Total -Lushnja	2024	64	55	68	68	

During the study period, in both complexes were realized respectively 6th and 7th of serological tests with interval from one test to another every 2 months. The level of sensitivity and specificity of the RB test was 95% and 92% and RA test was 81% and 75%, used for monitoring of brucellosis in cattle, compared with iELISA test, were considered in good level and approximate with the results obtained by various authors. Based on the evaluation criteria of sensitivity and specificity, RB serologic test has an excellent compatibility with "gold standard" iELISA test. Based on this argument emphasize that monitoring of the epidemiological situation of brucellosis always considered complete if screening tests are followed by a test "gold standard" as iELISA or CF, allergy test and bacteriological test [5].

3.2 Isolation, identification and characterization of *Brucella* spp. in aborted cattle.

Colonies of strains of *Brucella abortus* and *Brucella.melitensis*, isolated on solid mediums, presented the following morphological features:

transparent and convex colony with smooth surfaces and sides, round and with 0.8 - 1.5 mm size.

Brucella generally doesn't require more conditions for growth in cultural mediums, but we found that for the initial isolation it's necessary Farrell medium use, and the presence of Selective Supplement *Brucella* in different mediums. *Brucella* species are usually catalase and oxidase positive, reduce nitrate to nitrite and H₂S production [1]. Characterization at biovar level was conducted with sera-agglutination test with mono-specific A and M serums. For reconfirmation was sent to the Veterinary Laboratories Agency (VLA), London. This institution identifies isolates as strains *Brucella abortus* biovar 6 and *Brucella melitensis* biovar 3.

Although we worked with a limited number of cattle, notwithstanding the large number of samples analyzed in our study were isolated in 100% of analyzing cattle the cause of brucellosis in aborted and sera-positive cows. From pathological material in cows from which *Brucella* was isolated, were: 100% of supra mammary lymph nodes, spleen and content of abomasum [3].

Table 2: Biochemical characteristics and growth in colored mediums of isolates in the study.

Samples:	Characteristics of growth						Ac	Mono-specific serums		Interpretation
	Urea	H ₂ S	CO ₂	O ₂	BF	TH		A	M	
Durres	+	+	+	+	-	+	-	+	-	B.abortus 6
Lushnja	+	-	-	+	+	+	-	+	+	B.melitensis 3

3.3 Control of brucellosis through vaccination and vaccination schemes.

Brucella abortus is the main etiological agent of brucellosis in cattle, while *Brucella melitensis* is the main etiological agent of brucellosis in small ruminants, but despite this specificity, generally is recognized their migratory capacity in different animal species.

Generally prevent of infection and consequently disease prevention is achieved through the use of the vaccines. Basically, to access a better effectiveness, in the intensity and duration of immunity against intracellular agents, in order to encourage cellular mediators of the immune mechanisms are used live vaccines.

Control program is based on: serological testing, eliminating positive animals through slaughter and vaccination of uninfected animals. The main objective of this program is reducing the prevalence at acceptable levels with impact both in human and animal health, production with ultimate order the eradication of this infection.

Based on experimental studies in our working experience and of the many other countries [6], as well as in Directive 2002/598 /of European Commission, for healthy cattle from complexes in Durres and Lushnja we have supported precisely in these elements of control / eradication strategy of brucellosis:

- Serological investigation in every 2 months in all the cattle available in the complex; the immediate elimination of positive cattle; vaccination of cattle with the vaccine produced by the vaccine strain *Brucella abortus RB 51*, as a negative result of the whole complex in the last two tests, in the interval every 2 months and the increase of the level of bio-safety in the complex.

In the two complexes are vaccinated 1.411 cows, calf heifer, to aged over 4 months. Based on age, physiological and health condition as well as in the use of this vaccine scheme, with one intervention are vaccinated 1,411 cattle, with two interventions are vaccinated 729 cattle, while with three interventions are vaccinated 240 cattle.

Before and after application of the vaccine and at intervals of every 3, 6, 12 and 18 months after vaccination, for a period of 24 months to identify the absence or presence of anti-LPS antibodies were performed serological testing with RB and iELISA tests, which have resulted negative, but also to evaluate immunogenicity skills of the vaccine. Identification of vaccinated animals and the study of the mediators kinetic of the cellular immunity was realized with brucellina PPD produced by us. In the cattle complex in Durres was carried out the study of the skin thickness in cows after vaccination (mm).

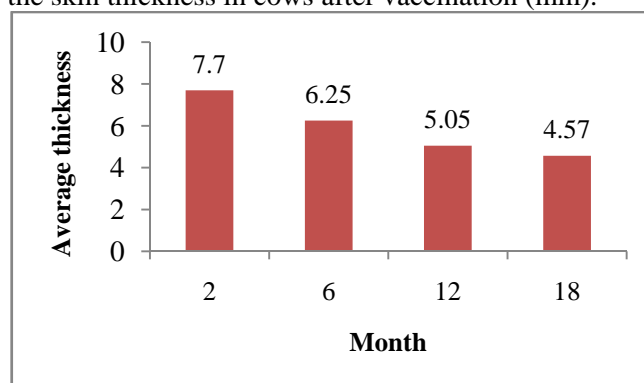


Figure 1: The skin thickness in cows after vaccination.(mm)

All vaccinated animals 100% reacted positively to the intradermal inoculation of 0.1 ml brucellina PPD after 2, 6, 12 and 18 months of the vaccination and the average of the mediators kinetic of the cellular immunity was 7.7 ; 6.25; 5, 05 and 4, 57 mm, indicating good protection levels induced by *Brucella abortus RB51* vaccine. The use of *Brucella abortus RB51* vaccine after cattle complexes were considered "free" by brucellosis and application of vaccination schemes associated with serological monitoring and enhancing of bio-safety level in cattle complexes not only eliminated abortions and the incidence of the infection, but 22 months after its application, complexes were considered practically healthy, as in two last serological controls carried out with test iELISA resulted negative.

Comparative studies in immunogenicity setting of the vaccines produced by strains of *Brucella abortus RB51* and *S19* realized in female vaccinated

calves aged 3, 5, 7 and 10 months infected with virulent strain, have confirmed that the vaccine *RB51* and *S19* protect animals respectively from infection and abortion 87% and 95%. In control animals was observed a higher incidence of infection associated with a high level of abortions. From this study it is concluded that there is no significant difference in vaccine effectiveness connected with the age when it is applied the vaccine [2]. Similar results with our results have taken the authors [6]. Based on pathogenesis, nature and transmission of infection as well as studies of our authors and more international authors, during our working despite the real conditions we have realized and have recommend vaccination of calves at the age of 4 to 5 months

4. Conclusions:

- The-vaccine produced by *B. abortus RB51* strain should be used to control the disease in endemic areas of infection or be an integral part of the strategy of eradicating of the disease [6].
- Given that the vaccine strain does not promote the production of agglutinin, vaccine application allows veterinary structures to carry out epidemiological monitoring of brucellosis in cattle because the vaccine does not interfere with serological tests.

5. References

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