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Seroprevalence of Bovine Brucellosis in Albania Dairy Cattle. Impact of Test and Slaughter Strategy in Herd Prevalence

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Abstract:

Background: *Bovine brucellosis in dairy cattle is an important bacterial disease and possesses a high risk for both human and animal. Bovine brucellosis is mainly caused by Brucella abortus which occurs worldwide and is present in Albania. Cattle are susceptible to B. abortus, B. melitensis and B. suis. In Albania, bovine brucellosis is detected by active and passive surveillance. In 2016, the active surveillance was focused on dairy farms with more than 20 animals/farm, which in 2018 is extended to the farms larger than ten milking cows. According to new unpublished studies, both dairy and beef cattle are mostly affected by B. abortus. The aim of this study was to assess herd and within herd prevalence of bovine brucellosis in*

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dairy cattle and to evaluate the national bovine brucellosis control program based on serological results of 2016 and 2018.

Materials and methods: *Bulk milk samples were collected from dairy farms. The milk samples were analysed in parallel by a milk ring test assay and ELISA test. Sera from individual animals in milk ring test and milk ELISA test positive herds were screened by Rose Bengal Test and positive results were confirmed by the ELISA test.*

Results: *Fourteen out of 492 dairy herds tested positive in 2016, compare to fifteen out 751 dairy herds in 2018. The herd prevalence dropped 33.3% in 2018 (1.9%) compare to 2018 (2.9%), while number of positive animals declined by 29% in 2018 compare to 2016 results.*

Keywords: Bovine Brucellosis, herd prevalence; zoonotic disease; Control program.

1. INTRODUCTION

In Albania, Brucellosis is considered one of most important infectious disease in ruminants and it is endemic. In addition, it is a zoonotic disease and all ages are susceptible to infection [10, 11]. Out of twelve medical significant known brucella, both *B. melitensis. B. melitensis* are isolated and identified in Albania from small and large ruminants [4]. *B. melitensis,* naturally is associated with goats and sheep brucellosis, while *B. abortus* is adopted to cattle and cause bovine brucellosis [9, 11]. Both species could cause cross-species infection, however the main hosts serves as reservoir of infection. Geographically, bovine brucellosis has a worldwide distribution, while sheep and goat brucellosis have a limited spread in certain areas [6, 7, 9]. The disease status of animals at farm and individual level differ highly between countries and is directed related to type of strategies in place. The main route of transmission brucellosis from infected herds to the free herds is by uncontrolled and illegal movement of infected animal [4, 7, 9]. Level of biosecurity measures, particularly animal management play a significant role in Brucellosis transmission. Co-grazing of cattle and small ruminants, as it is a

common practice to the Albanian farms, is a great risk factor for transmission of *B. melitensis* from infected sheep and goats to cattle and or *B. abortus* form cattle to sheep and goats [9, 10, 11]. The main clinical sign of brucellosis is abortion of infected animals [4, 6, 7, 9]. The abortion occurs only for the first time after infection and host adopted *Brucella species* cause abortion storm, while infection with non-adopted species cause sporadic abortion [4, 7,9]. Infection with *Brucella* spp. has a typical latent course, which make it difficult to detect infected animal. In female animals, the organism localizes in the mammary lymph nodes and mammary glands of 80% of infected animals, and these continue to excrete the pathogen in milk throughout their lives acting as carriers but intermittently [9]. The control of brucellosis is based on vaccination, test and slaughter or combination of them. Active and passive surveillance of brucellosis play an important role in control of bovine brucellosis.

Brucella antibodies are present in blood and also in milk. The pool milk samples from milk could be tested in order to evaluate the herd health status. A most wide used, simple, fast, cheapest and sensitive method is Milk Ring Test (MRT) which could be used in bulk milk samples. MRT was first described by German scientist Fleischhauer [2] and it is widely used as a herd test to know the prevalence of *Brucella* infection and for screening the herd. In addition, MRT can also be used to test individual milk samples. Along the several advantages the MRT has a main disadvantage related to specificity, it may give false-positive results shortly when the sample is colostrum (immediately after parturition), at the end of lactation period and when mastitis present [1]. The MRT positive herd must be closely investigate by performing individual screening test and confirmatory tests.

A range of serological tests are widely used for the diagnosis of bovine brucellosis, while in Albania Milk Ring Test (MRT), Milk ELISA Test, Rose Bengal Test are used as screening test, while Complement Fixation Test (CFT), competitive ELISA and Indirect ELISA tests are officially in use as confirmatory Tests. Recently, Fluorescence Polarisation Assay is available at infectious disease laboratory of Veterinary Faculty which may be running as screening test on both milk and sera samples and as confirmatory test on sera blood samples [4]. In Albania since 2016, there is in place an active

surveillance program for control of bovine brucellosis (BBCP). The program aims to assess the herd prevalence and within herd prevalence by using strategically screening tests with confirmatory test on commercial dairy farms. In this study we present the results of RMT, Rose Bengal Test (RBT) and ELISA confirmatory test for bovine brucellosis in 2016 and 2018 [2, 3, 11]. In addition, by comparing serological results, the impact of current strategy will be evaluating, and rational conclusions may be draw for improving BBCP.

2. MATERIALS AND METHODS

As a sample we used milk and blood from cows belong to farms included in the national surveillance programme for bovine brucellosis, according to the approved framework (Figure 1). Identification of dairy herds that were included in this study was based on the RUDA system, and a surveillance plan was carefully designed and followed by veterinary authorities and supported by the PAZA EU-funded project. Bulk milk samples were collected and sent to the national reference laboratory, the Food Safety and Veterinary Institute (FSVI), Tirana and tested by MRT and milk ELISA tests. The positive herds were followed up, bleed all animals older than 12 months and sera blood samples were tested by the Rose Bengal Test. Positive samples were then tested by ELISA test.

2.1 Milk Ring Test (MRT)

The MRT was performed by adding 30 µl of antigen to a 1 ml volume of whole milk that had been stored for at least 24 hours at 4°C. The height of the milk column in the tube was at least 25 mm. The milk samples were not frozen, heated or subjected to violent shaking. The milk/antigen mixtures were incubated at 37°C for 1 hour, together with positive and negative control samples. A strongly positive reaction was indicated by formation of a dark blue ring above a white milk column. Any blue layer at the interface of milk and cream was positive as it might be significant, especially in large herds. The test was considered to be negative if the colour of the underlying milk remains homogeneously dispersed in the milk column. If the milk at

the bottom of the tube became gradually whitened, the result was regarded as inconclusive and the test was repeated.

2.2 Rose Bengal Test

The Rose Benga Test was perfomet on bovine sera, 30 µl serum is mixed with an equal volume of antigen on a white plastic plate to produce a zone approximately 2 cm in diameter. The mixture is rocked gently for 4 minutes at ambient temperature, and then observed for agglutination. Any visible reaction is positive, while absence of agglutination is negative [4, 6, 7, 9].

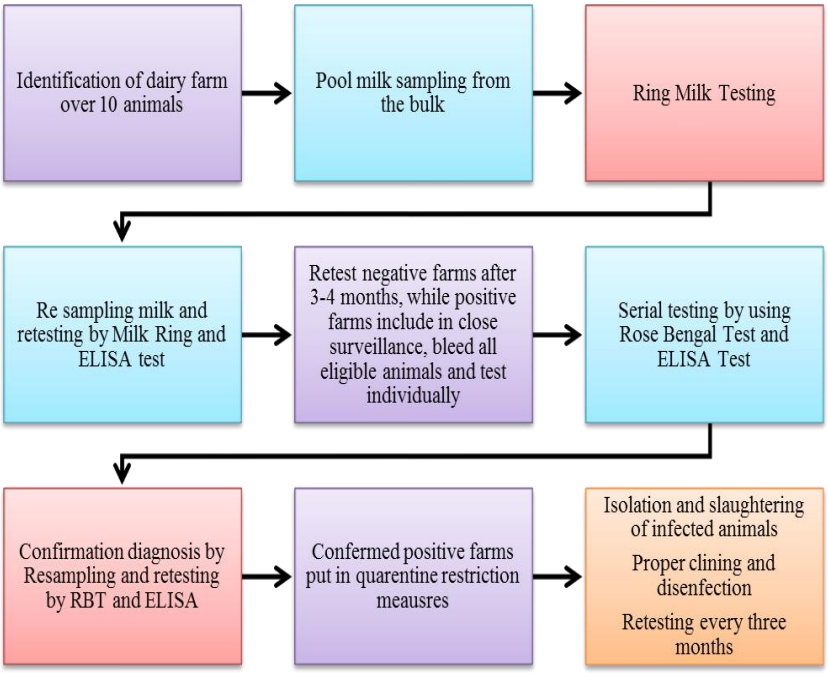
2.3 ELISA method

Individual sera blood samples were tested by using Brucellosis Antibody Test Kit produced by IDEXX company. The test kit supplies microplates coated with *Brucella abortus* lipopolysaccharide antigen and identify presence of specific IgG in blood sera. The test was run according to the manufacturer's instruction. The samples and negative control were run as single, while the positive control was run in duplicate. The optical density (OD) for all positive control, negative control and each sample were read by using ELISA reader at 450 nm wave length. For positive control the OD mean value was calculate. The classification criteria for animal health status were based on S/P value. S/P value was calculated by formula:

$$S/P = \frac{(\text{OD of sample} - \text{OD negative control})}{(\text{OD positive controls mean}) - \text{OD negative control})} * 100$$

Table 1. Criteria used for brucellosis animal's status based on ELISA results

Animal status	Positive	Doubtful	Negative
S/P value	≥120%	110-120%	≤110



3. RESULTS AND DISCUSSION

Serological results are presented in Tab. 2 and Tab. 3, and Figure 1 and 2.

3.1. MRT results

The herd prevalence of bovine brucellosis was 3.96% and 1.95% in first and second monitoring phase respectively, while average herd prevalence was almost 3%. MRT is considered as a suitable method for detecting infected herds, however it is known from very early studies that false positive reactions may occur in colostrum or milk at the end of the lactation period and milk from cows suffering from a hormonal disorder or mastitis. In addition to good sensitivity the MRT has several advantages such as simplicity, wide acceptability, cost effectiveness and non-invasive sampling. Those make it as suitable

preliminary screening test of bovine brucellosis. Two out of 16 MRT positive herds revealed negative results in Rose Bengal Test which indicate a specificity 87.5%, which closer with other studies. The MRT reported to have a sensitivity of 89%. Recently, Salman *et al*. (2012) found similar levels of sensitivity and specificity for MRT which were 85% and 95%, respectively [4, 6, 10].

Table 2. Mil Ring Test and ELISA test results on 492 dairy herds and 751 dairy herds

Year	Number of farms tested	Number of positive farms	Number of positive farms	Number of negative Farms MRT/ELISA
		Screening test (herd prevalence)	Confirmatory tests (herd prevalence)	
2016 Herds > 20 animals	492	16 (3.3%)	14 (2.99%)	476/481
2018 Herds > 10 animals	751	39 (5.2%)	15 (1.99%)	712/736

Table 3. Comparing Rose Bengal Test and ELISA Tests results on MRT positive herds involved in BBCP

Type of serological	Year	
	2016	2018
Brucella positive cattle based on Rose Bengal Test results	133	95
Brucella positive cattle based on Complement Fixation Test and ELISA Test	133	95

The prevalence of bovine brucellosis in MRT positive farms was 21.2%. The positive samples in RB test were confirmed either by ELISA test. The agreement between RB and c –ELISA was ideal, all positive samples in RBT were positive in confirmatory test. The exceptionally agreement may be explained that all positive sera gave a high degree of agglutination in Rose Bengal Test [5, 7, 8].

Further work is ongoing such as continuing of bovine brucellosis surveillance, stamping out of positive animals, farmer's compensation, proper application of cleaning and disinfection of infected premises, identification of *Brucella* species that affect cattle and based on that drafting proper control strategy at national level.

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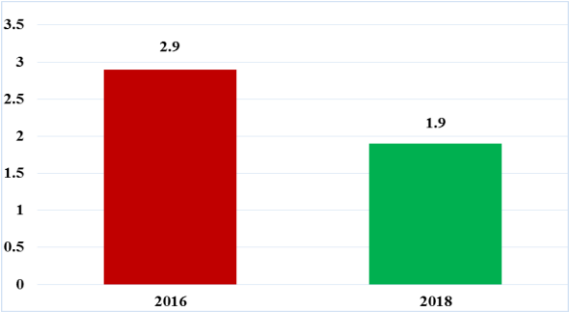

Figure 2 – Herd prevalence of bovine brucella according serological results, in 2016 and 2018 respectively. It is a significant reducing of prevalence in 2018.

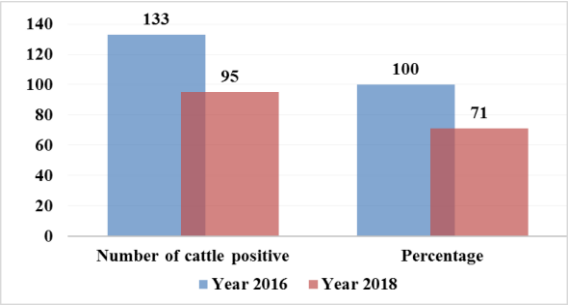

Figure 2 – Number of brucella positive cattle in framework of BBCP during 2016 and 2018 respectively. It is a significant reducing of positive animals in 2018, as results of application test and slaughter policy.

4. CONCLUSIONS

Our results indicate that bovine brucellosis in Albania is present in dairy farms at relatively low prevalence level. Herd prevalence of bovine brucellosis according MRT results was approximately 2.9% in 2016 and 1.9% in 2018. The herd prevalence rate decrees by almost 1/3 only two year since bovine brucellosis control program is in place. Number of positive animals to bovine brucellosis detected in 2018 dropped at 29 % compare with 2016. Our findings indicate that BBCP is playing an important role in controlling of bovine brucellosis in animals, and indirectly in human. This successful approach may be useful for extending it in other cattle categories, especially in small dairy farms.

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