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Preliminary results of a serological survey of bovine brucellosis in Albania

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Abstract

Bovine brucellosis is an important bacterial zoonotic disease caused by *Brucellaabortus*. It 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. Currently, limited data is available on the causal agent of the disease in Albania.

The aim of this study was to determine the herd and individual animal prevalence of bovine brucellosis in dairy farms with more than 20 cattle.

Materials and methods: Bulk milk samples were collected from dairy farms. The milk samples were analyzed by a milk ring test. Sera from individual animals in milk ring test positive herds were tested by Rose Bengal test and positive results were confirmed by the complement fixation test.

Results: Eleven out of 278 herds were positive in the first monitoring round according to the milk ring test; nine of these herds were positive in Rose Bengal and complement fixation test. In the second phase, five of 257 dairy farms were positive in milk ring test and subsequent serological tests. In the milk ring test positive farms 5.2 to 59.3% of cattle were affected by brucellosis.

Keywords: Zoonotic disease outbreak; Bovine Brucellosis; Ring Milk Test.

1. Introduction

Brucellosis is one of most important infectious disease in ruminants in Albania, it is considered to be endemicandcattle ofall ages are susceptible to infection. In addition, it is a severe zoonotic disease [6, 7]. Out of more than eight medical significant known ***Brucella*** species, the two most important species for Albania are *B.abortus*an*B. melitensis. The latter* is usually associated with goats and sheep brucellosis, while *B. abortus* is adapted to cattle and causes bovine brucellosis [5, 7]. Both species could cause cross-species infection, however, the main hosts serves as reservoirs of infection. Bovine brucellosis has a worldwide distribution whereas sheep and goat brucellosis has a limited distribution in certain areas, particularly in Mediterranean countries. The disease status of animals at farm and individual level differs considerably between countries and is influenced by the types of strategies in place and in general veterinary services functionality. The main route of transmission brucellosis from infected herds to the free herds is by uncontrolled and illegal movement of infected animal. The level of biosecurity measures, particularly animal management, plays a significant role in brucellosis transmission. Co-grazing of cattle and small ruminants, is a common practice on

Albanian farms, which is a great risk factor for transmission of *B. melitensis* from infected sheep and goats to cattle and or *B. abortus*from cattle to sheep and goats [5, 6, 7]. The main clinical sign of brucellosis is abortion in infected animals. The abortion occurs only the first time after infection when host-adapted *Brucella*speciescan cause abortion storms. Infection with non-adaopted species usually cause sporadic abortion. Infection with *Brucella*spp. typically has a latent course, which makes it difficult to detect an infected animal. In dairy animals, the organism localizes in the supra-mammary lymph nodes and mammary glands of 80% of infected animals, and these continue to excrete the pathogen in milk intermittently throughout their lives acting as carriers [5]. The control of brucellosis is based on vaccination, test and slaughter or combination of these strategies. Active and passive surveillance of brucellosis play an important role in control of bovine brucellosis. Several serological tests are widely used for the diagnosis of bovine brucellosis.

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

In Albania there is no strategy for control of bovine brucellosis at national level, however there is in place an active surveillance programme for the disease. The programme aims to assess the herd prevalence and within herd prevalence by means of strategically screening tests with confirmatory test on commercial dairy farms. In this study we present the results of MRT, Rose Bengal Test (RBT) and confirmatory test for bovine brucellosis [6].

2. Materials and Methods

We used milk and blood from cows belong to farms included in the national surveillanceprogramme for bovine brucellosis, according to the 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 EU-funded project. Bulk milk samples were collected and sent to the national reference laboratory, the Food Safety and Veterinary Institute (FSVI), Tirana. The positive herds were followed up and venous blood was collected from eligible animals and sera blood samples were tested by the Rose Bengal Test. Positive samples were then tested by either the CFT or ELISA test.

2.1 Milk Ring Test (MRT)

The test 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 considered to be 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.

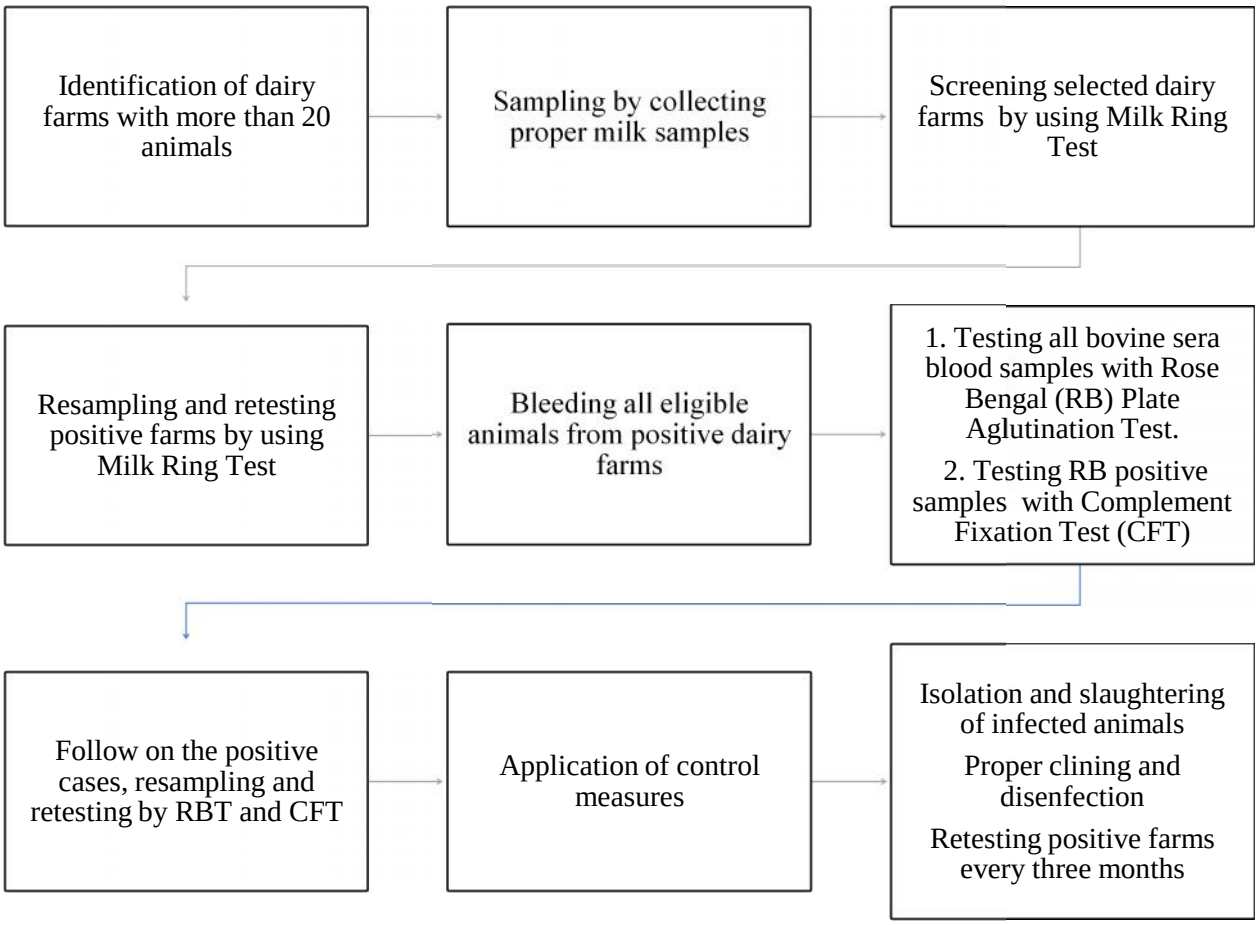

Figure 1: Scheme to show the process steps in mounting surveillance of bovine brucellosis.

2.2 Rose Bengal Test

Serum (30 µl) was mixed with an equal volume of antigen on a white tile or enamel plate to produce a zone approximately 2 cmin diameter. The mixture was rocked gently for 4 minutes at ambient temperature, and then observed for agglutination. Any visible reaction was considered to be positive [5].

2.3 Complement Fixation Test

The Complement Fixation Test (CFT) was used to test positive Rose Bengal samples according to the ISUV standard operating procedure (SOP) which was based on OIE guidelines and EU requirements.

Briefly, sera samples were diluted from 1:2 to 1: 512, mixed together with antigen in the presence of complement and allowed to develop. Results were expressed in international CFT unit (ICFTU), calculated in relation to those obtained in a parallel titration with a working or national standard serum calibrated against the OIEISS, which contains 1000 ICFTU. The sera that gave a positive fixation at a titer equivalent to 20 ICFTU or greater, were classified as positive samples [5, 7].

2.4 ELISA method

Individual sera blood samples were tested by using Brucellosis Antibody Test Kit (IDEXX, Country). The test kit supplied microplates coated with *Brucellaabortus* lipopolysaccharide antigen and was used to identify the presence of specific IgG in blood sera. The test was run according to the manufacturer's instruction. The samples and negative control were run singly, while the positive control was run in duplicate. The optical density (OD) for all positive controls, negative controls and each samples

were read by an ELISA reader at 450 nm wavelength. For the positive control the OD mean value was calculate. The classification criteria for animal health status was based on S/P value, which was calculated with the formula:

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

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

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

3. Results and Discussion

Serological results are presented in Table 2 and Table 3; while the herd health status for bovine brucellosis is presented in Table 4.

3.1. MRT results

The herd prevalence of bovine brucellosis was 3.96% and 1.95% in first and second monitoring phases, respectively, while average herd prevalence was almost 3%. The 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: it is widely acceptable, cost effective and involves non-invasive sampling. These attributes make it a 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 is closer with other studies. The MRT is reported to have a sensitivity of 89% [4]. Recently, Salman et al. (2012) found similar levels of sensitivity and specificity for MRT which were 85% and 95%, respectively [8].

Table 2. Mil Ring Test results of 497 dairy farms

Phase	Number of farms tested	Number of positive farms (herd prevalence)	Number of negative Farms
First phase	278	11 (3.96%)	267
Second phase	257	5 (1.95%)	214
Total 2016	**535**	**16 (2.99%)**	**481**

3.2. Results of Rose Bengal (RB) and Complement fixation (CF) Tests

In general, the RBT and MRT have been shown in other studies to have high sensitivity but lower specificity. The MRT is not normally used on individual animals because of the higher rate of false positives (less specificity) [5, 7].

The MRT and RBT are generally useful as screening test for brucellosis, especially in developing countries where other tests are cumbersome to perform on a large scale and/or require special equipment and expertise, these tests still have major limitations where vaccination or detail records are not available. As a result of these limitations, other confirmatory tests e.g. ELISA, CFT, SAT, FPA (fluorescence polarisation assay) must be carried out in conjunction with MRT and RBT in order to confirm the brucellosis status [5, 7].

Table 2. Rose Bengal Test, Complement Fixation Test and ELISA Tests results of sera blood samples from MRT positive farms.

Serological test	Number of serum samples tested	Number of positive sera	Number of negative sera
Rose Bengal Test	626	133	493
Complement Fixation Test	74	74	74
ELISA Test	59	59	59
Total 2016	**626**	**133**	**493**

The prevalence of bovine brucellosis in MRT positive farms was 21.2%. The positive samples in RB test were confirmed either by CFT and ELISA. The agreement between RB and CFT and ELISA was very high, all positive samples in RBT were positive in CFT as well. The exceptional agreement may have been because all positive sera gave a high degree of agglutination in Rose Bengal Test [5, 7, 8].

Table 4. Herd health status include in study according serological tests results

	Number of farms tested	Number of positive farms	Number of negative Farms	
Ring Milk tests	497	16	481	
Confirmatory tests	**16**	**14**	**2**	

Further work is ongoing, such as continuing of bovine brucellosis surveillance, elimination 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 an appropriate control strategy at national level.

4. Conclusions

Bovine brucellosis is quite widespread in dairy farms in Albania. Bovine brucellosis prevalence between herds according MRT results was approximately 3%: there were 4% positive in the first monitoring round and 2 % in the second phase. The milk ring test is a simple procedure for screening herds for bovine brucellosis, its specificity was 87.5% and false positivity was 12.5%. Only two farms produced false negative results in MRT.

The prevalence of brucellosis in the positive MRT farms at individual level was 21.2%, while the within farm prevalence ranged from 5.2-59.3% (data not shown), which indicates the circulation of *Brucella*spp. for quite long time inside the farms.

These results strongly suggest the urgent need for drafting a rational strategy to control bovine brucellosis in Albania.

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