German Journal of Veterinary Research

eISSN:2703-1322

Short Communication

Bovine brucellosis serological survey in small dairy herds in Lushnja district, Albania

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This article is published in the special issue: Brucellosis from a One-Health Perspective

Article History:
Received: 25-Mar-2022
Accepted: 10-May-2022
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Abstract

Bovine brucellosis is an important zoonotic disease in Albania. Both *B. abortus* and *B. melitensis* have been isolated from ruminants. National control and eradication programs for brucellosis are applied on sheep and goat farms and larger dairy cattle farms. However, the current control programs for brucellosis do not cover small dairy cattle farms, and there is no valid data on the prevalence of the disease in this category; this prevents formulating evidence-based and effective strategies for control of the disease in this population subset. Therefore, the current study aimed to assess the herd and within-herd prevalence of bovine brucellosis in small bovine herds and provide scientific evidence for establishing an evidence-based approach to control the disease in this subset of the population not previously included in the national eradication program. To achieve this objective, a statistical survey was designed and implemented in small dairy herds in the Lushnja district, where samples from statistically selected herds were serologically tested in parallel with Rose Bengal Test (RBT), Fluorescence Polarization Assay (FPA) and Enzyme-Linked Immunosorbent Assay (ELISA). In total, 120 dairy herds were randomly selected from a list of 1,955 registered herds: from these selected herds, 368 blood samples were collected from all animals older than 12 months, and their sera were tested using RBT, FPA, and c-ELISA. The test results revealed no positive or suspect cases. Based on these results, we are confident ($P \leq 0.05$) that *Brucella* spp is not circulating in this subpopulation of cattle in the Lushja district. This deduction is supported by analyses of the main risk factors, other epidemiological data, and the perceptions of official and private veterinarians. This is the first structured survey of bovine brucellosis in small dairy herds in Albania. In conclusion, our study results and our findings show that the epidemiological status of bovine brucellosis in the Lushnja district is encouraging. Therefore, a test and slaughter control program appears appropriate in smaller herds. Furthermore, the approach used in this pilot study could be extended to establish the prevalence of brucellosis in other districts, the result of which would establish the basis for rational control measures in the smaller herds of cattle.

Keywords: *Brucella abortus*, Small dairy herd, Bovine brucellosis, Serology, Albania

Citation: Arla, J., Gerald, M., Anita, K., Luigj, T. and Xhelil, K. 2022. Bovine brucellosis serological survey in small dairy herds in Lushnja district, Albania. Ger. J. Vet. Res. 2 (1): 31-35.https://doi.org/10.51585/gjvr.2022.1.0038

Introduction

Brucellosis is a highly infectious zoonosis that causes severe disease in humans and has a significant economic impact on ruminant animals (EU, 2008; Quinn et al., 2011; EFSA, 2015). People can acquire infections by consuming unpasteurized milk and dairy products originating from infected animals (Piao et al., 2020). Brucellosis may be suspected usually based on prominent indicators such as the occurrence of a storm of abortion in animals and undulant fever in human cases, but its confirmation is made through laboratory diagnosis (Quinn et al., 2011). The strategy for brucellosis control should be designed considering the general guidance and principles listed in the appropriate European Union (EU) and World Organisation for Animal Health (OIE) standards. However, it must be adapted according to disease status, prevalence, and a range of aspects related to country specificities.

In general, control of brucellosis is based on vaccination and surveillance program. Standard available vaccines for cattle are live attenuated vaccines based on *B. abortus* S19 and *B. abortus* RB51 vaccinal strains, while *B. melitensis* Rev 1 strain remains the standard vaccine strain used in live attenuated vaccines for sheep

and goats (EU, 2008; Quinn et al., 2011; EFSA, 2015; OIE, 2018). In countries with low disease prevalence, a test and slaughter policy is reported as a rational strategy that aims to eradicate the disease (Wareth et al., 2019). The experiences showed that milk pasteurization and controlling the disease in susceptible animals could reduce human brucellosis risk. This makes brucellosis one of the good examples where one health approach can help to control disease in animals and prevent zoonotic risk (EU, 2008; Quinn et al., 2011; EFSA, 2015; OIE, 2018).

In Albania, both *B. abortus* and *B. melitenis* are identified in ruminant animals (Bruce et al., 2014; PAZA Project, 2015; Fero et al., 2018; Mersini et al., 2019; Fero et al., 2020). The national Bovine Brucellosis Control Program (BBCP) was initiated five years ago (PAZA Project, 2015, 2016; Fero et al., 2018). The program started with screening all dairy cattle herds larger than 20 animals, while in subsequent years, the program expanded to the herds with >10 milking cows. The strategy was drafted and monitored by an EU-funded project and national experts. It was based on the use of the Milk Ring Test (MRT) to screen the herds, followed by individual retesting of all animals from the MRT positive herds with the Rose Bengal Test (RBT) and the Enzyme-Linked Immunosorbent Assay (ELISA) test. All individual animals from the MRT-positive herds which tested positive for RBT and ELISA were considered infected. Data analysis showed that the prevalence of bovine brucellosis is low in dairy cattle herds with >10 milking cows and high in the beef cattle herds (PAZA Project, 2015, 2016; Fero et al., 2020).

The Bovine Brucellosis Control Programm (BBCP) foreseen that before expanding the program to smaller herds, a serological survey should be carried out to assess the herd prevalence and individual animal prevalence. Therefore, a serological survey was performed in the county of Fier focused on small dairy herds (3–9 milking cows) to assess both herds and within-herd prevalence of brucellosis in this bovine population.

Materials and Methods

Sample size

To provide statistically valid information, there were set following parameters: to maximize the tested animals we set a prior prevalence 50% (p =0.5), confidence 95% (z = 1.960) and accepted error or precision (±10%; d = 0.1), q =1-p (0.5). The minimum number of small bovine herds to test to suffice these statistical parameters is 96 (Formula 1). The formula for correct calculation of sample size at farm level (minimal number of farms to be sampled):

$$n = \frac{z^2 + p * q}{d^2} = \frac{1.96^2(0.5 * 0.5)}{0.1^2} = 96$$

Definition of the sampling frame

From a list of 1,955 herds, we randomly selected 120 herds with 3-9 animals per herd. All animals older than 12 months in the chosen herds were sampled on the spot. The selected 120 small dairy herds are in 52 villages out of 117 total villages of the Lushnja district, a part of the county of Fier. The average size of 120 selected herds is 3.1 animals per herd. In total, 368 cows were sampled at the farm by applying animal welfare precautions. From the coccygeal vein of each animal, approximately 9 ml of blood was collected in the plane tube test. The collected blood samples were left to clot for 30-60 minutes before submitting to the laboratory and left overnight at 4-80°C. Sera samples were harvested and kept at -20°C until they were tested. All serum samples were tested at the Infectious Disease Laboratory at the Faculty of Veterinary Medicine, Agricultural University of Tirana. All collected sera were tested in parallel.

Serological examination

All sera were first tested with the Rose Bengal Test (RBT), followed by Fluorescence Polarization Assay (FPA) and Enzyme-Linked Immunosorbent Assay (ELISA). The sample was considered positive when it gave a positive reaction to RBT and one or both other tests (FPA and ELISA) (OIE, 2018).

The RBT was performed according to laboratory Standard Operating Procedures (SOP) based on the OIE manual. Briefly, the sera and antigen were left to reach room temperature before use. Then, equal volumes (30 μL) of standardized *B. abortus* antigen and test serum were mixed thoroughly and rotated on a white plastic plate using a stick applicator for 4 minutes. Any appearance of agglutination was recorded as a positive sample, and according to the degree of agglutination, positive samples were classified as strong positive to weak positive samples, ranging from one (+) to four plusses (++++). The samples which do not show agglutination within 4 minutes were judged as negative (-).

ELISA from (IDEXX) was performed on a 96-well polystyrene plate precoated with purified *Brucella abortus* lipopolysaccharide (LPS) antigen. A multi-species horseradish peroxidase (HRP) was used, and a substrate solution was added after washing to eliminate excess conjugate. The coloration of the antigen-antibody conjugate-peroxidase complex formation depended on the number of anti-*Brucella* antibodies that were present in the specimen tested. Thus, in the presence of antibodies, a blue solution appeared, which became yellow after adding the stop solution, while in the absence of antibodies, no coloration appeared. Both negative and positive controls were run in duplicate. The results of the ELISA tests were expressed as the value of the sample (S) divided by the value of the positive control serum (P) supplied in the IDEXX ELISA kit, as determined by measurement of the optical density (OD450) with a "TECAN" ELISA plate reader (Tecan Austria GmbH, Grödig, Austria).

$$S/P\% = \frac{SampleA(450) + NC\overline{x}}{PC\overline{x} - NC\overline{x}} \times 100$$

Table 1: Number of animals for each test and the health status.

Tested method	Tested samples	Animal health status
Rose Bengal Test (RBT)	368	Negative
Enzyme-Linked Immunosorbent Assay (ELISA)	368	Negative
Fluorescence Polarization Assay (FPA)	368	Negative

Table 2: Probability of animals tested positive.

Tested herds	Positive herds	Overall herd prevalence	SE[1]	95% CI (lower)	95% CI (higher)[2]
120	0	0%	0.02	0%	4%

[1]SE: Standard error.

[2]CI: Confidence interval. In any case, the overall herd prevalence did not exceed the 4% level.

Where; Sample A (450) = Sample Optical Density, NC$\overline{x}$ = mean value of negative control optical density, and PC$\overline{x}$ = mean value of positive control optical density).

The criterion used for determining the status of animals tested was the S/P value: an S/P value <110% was considered negative. When the S/P value was 110–120, the results were considered inconclusive, and an S/P value greater than 120 was considered positive. The FPA is a simple homogeneous assay and is very rapid. It was run according to the manufacturer's instructions (*B. abortus* antibody test B1001 KIT. Ellie Headquarters Milwaukee, U.S.A Patent No. 5,976,820; 1999). All samples and reagents were allowed to reach room temperature. The sample diluent was prepared by diluting it with distilled water at a 1:25 ratio. Briefly, the test procedure was performed in 10×75 mm borosilicate glass test tubes for single tube FPA instrument glass tubes; 20 μl of samples and controls in 1 ml of diluted sample diluent were pipetted. Negative controls were run in triplicate, while positive control and samples were run as single tests. After mixing, the samples were incubated (3-30 minutes) at room temperature, and a first (blank) reading was obtained using Sentry® Software 2.3.26.exe. Next, the tracer was added (10 μl) to all samples and controls. A second reading was taken after 2-5 minutes, and millipolarisation (mP) units were obtained.

The results of the FPA tests were expressed as delta mP (ΔmP) values of the samples and were calculated as the difference between the mP value of the samples and the average of the negative controls mP values. Animals that produced a titer under 10 ΔmP were considered negative, animals that showed a titer between 10–20 ΔmP were considered doubtful (suspicious or suspect), and animals that produced a titer higher than 20 ΔmP were considered positive.

Results and Discussion

In total, 368 animals from 120 farms were tested in parallel (Table 1). The criteria to assess the herd status are based on positive results from the ELISA and/or FPA tests. In addition, the animal's health status was judged according to laboratory results. Although there was no identification of any positive animal in either test, there could be a limited probability of having any positive animals (Table 2).

Regarding the biosecurity measures and animal health status, the dairy industry is believed to be well developed in the Lushnja District. However, studying the farm size structure shows that this sector is not as attractive as the potential is available. The farm size is smaller than assumed, and the total number of animals managed on farms, ranging from 1-2 animals, is 4758 animals, while in farms that own 3–9 milking cows, it is almost double, or 8476 animals, according to the national livestock and veterinary information system (RUDA system) (Table 3).

During the sample collection, information regarding history of abortion, cases of human brucellosis, type of farm management, presence of other ruminant animals, presence of chicken hens on the farm, use of animal movement certificate for buying replacement animals, use of animal movement certificate for selling the animals, animal identification, physiological status, animal age, important diseases present in the farm, and availability of disinfection point were collected (Table 4).

The current study is the first cross-sectional epidemiological study performed on this important cattle subpopulation. There was no detection of any positive animal in any test. However, the information provided by this study is helpful. We are more than 90% sure that this subpopulation is free of antibodies to *B. abortus* and or *Brucella* spp. infections. The main limitation of our study in terms of its applicability at the national level; however, it would be a case to suggest expanding the current BBCP used on farms larger than 10 milking cows to include farms with 3–9 milking cows and enforcing passive surveillance in the remaining subpopulation.

In this regard, monitoring abortion cases and human brucellosis will be critical for brucellosis control in general and particularly in this subpopulation. This approach is recommended to be utilized in larger geographical areas before expanding the active surveillance to the smaller farm subpopulations. In addition, for successful brucellosis control, we suggest undertaking and strengthening further measures, such as: continuously updating the national livestock and veterinary information system (RUDA system), improving animal identification, strengthening animal movement control, reporting, and investigating abortion cases, and implementing biosecurity measures.

Table 3: The size and number of animals on the tested farms according to the national livestock and veterinary information system (RUDA system).

Farm size	**Number of farms**	**Number of animals**
1 milking cow	1516	1516
2 milking cows	1621	3242
Subtotal farms 1-2 animals	3137	4758
3 milking cows	888	2664
4 milking cows	473	1892
5 milking cows	267	1335
6 milking cows	151	906
7 milking cows	100	700
8 milking cows	65	520
9 milking cows	51	459
Subtotal farms with 3-9 animals	1,995	8,476
Total	**5,132**	**13,234**

Table 4: The summarized results of biosecurity and animal status.

Biosecurity measures	**Number**	**Percentage (%)**	**Comments**
Human cases	2	-	There were samples from two additional farms that experienced human cases of brucellosis; however, no positive animals were detected. Therefore, those farms and 24 other additional farms are not included in the randomly selected farms.
Abortion	13	10.8	In almost all abortion cases, brucellosis was excluded. However, there is not in place any abortion surveillance program.
Presence of sheep on the farm	5	4.1	The sheep and goats were vaccinated against caprine and ovine brucellosis.
Presence of goats on the farm	7	5.8	
Presence of the chickens	120	100	The farmers keep chickens and other animals in the same place.
Buying replacement animal	11	9.1	The farmers buy animals from the local market without any special restrictions.
Selling replacement animals	25	20.8	The farmers sell the replacement animals to relatives or a known friend.
Providing animal certificates for replacement animals	2	1.6	In general, animal movement certificate issues are not used, but recently it is strictly in force.
Mean age of sampled animals	7.2	-	There were 5 animals over 12 years old.
Foot rot disease	37	30.8	The animal houses are not suitable for comfortable conditions. There is a lack of foot rot disease control programs, and the indoor system is the risk for higher prevalence.
Mastitis	41	34.2	There is a complete lack of a mastitis control program.
Respiratory	26	21.6	Mostly in calves, there is no vaccination program for respiratory diseases.
Gastrointestinal tract disease	32	26.6	Mostly in calves.
Availability of disinfection point	0	0	There is an abius lack of biosecurity measures application including disinfection bath at entry farm points.

Article Information

Acknowledgments. The authors would like to thank field veterinarians Safigjen Sila, Zamir Rapi, Vladimir Hatija, and Kledis Çela, as well as farmers for their engagement and support.

Funding. This work was supported in part by project no. Europe Aid/128304/C/SER/ AL, under EU contract number 2009/223-812. The funder had no role in study design, data collection, and analysis, decision to publish, or preparation of the manuscript.

Conflict of Interest. The authors have no conflict of interest to declare.

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