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Seroprevalence of Brucellosis in Albania,2004-2012

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Abstract: ***Brucellosis is a zoonosis, and the infection is almost invariably transmitted by direct or indirect contact with infected animals or their products. This is a crossectionalseroprevalence study including specimens referred to the Institute of Public health in Tirana, Albania from patients suspected for Brucellosis over the period 2004-2012. 4020 patients were included in the study. The mean age of patients was 40.2 (±12.4) years with a range 1-84 years. 71.5% of study participants were males and 28.5% females. 43.5% of the me were farmers. The seroprevalence of Brucellaspp. among participants was 19% (95% CI: 17.8–20.2). Seroprevalence was higher in females than in males (11.2% compared with 10.9%, P = 0.029). By age group, the highest seroprevalence was found in those 15-24 years at 26.9% (95% CI: 13.9–17.0), with the lowest in the 1-4 year age group at 4.5% (95% CI: 1.5–20.4).***

Keywords: seroprevalence, brucellosis, diagnosis, serologic assay

1. Introduction

Brucellosis is a zoonosis, and the infection is almost invariably transmitted by direct or indirect contact with infected animals or their products. It is an important human disease in many parts of the world, especially in the Mediterranean countries of Europe, North and East Africa, the Middle East, South and Central Asia and Central and South America (1).Brucellosis is caused by members of the *Brucella* genus. Transmission of infection to humans occurs through breaks in the skin, following direct contact with tissues, blood, urine, vaginal discharges, aborted fetuses or placentas(2).The most frequent symptoms of brucellosis are fever, chills or shaking, malaise, generalized aches and pains all over the body, joint and low back pain, headaches, anorexia, easy tiredness and general weakness (3).Several species of Brucella that are important to public health exist amongst which *B melitensis* and *B suis*are more virulent for humans than *B abortus* and *B canis*although serious complications can occur with any species of Brucella. Humans are infected either by direct contact with blood, placenta or uterine secretions of infected animals, through breaks in the skin, by inhalation or by ingestion of unpasteurized milk and other dairy products. It is known that unpasteurized milk is sold in several parts of Albania. Brucellosis is an occupational hazard to individuals engaged in certain professions such as abattoir workers, veterinarians, livestock farmers and herdsmen (4).

In the absence of culture facilitates the diagnosis of brucellosis relies on agglutination tests, such as, the Rose Bengal test, serum agglutination test, the antiglobulin or Coombs test, complement fixation test, and the recently introduced immunocapture test.

The Rose Bengal test is used as a screening test and positive results are confirmed by the serum agglutination tests (5)This agglutination test is based on the reactivity of antibodies against the smooth lipopolysaccharide. In the Rose Bengal Plate (RBPT) agglutination test the sensitivity is high (>99%) and false negative results are rarely observed. To increase the specificity the test may be applied to a serial dilution (1:2 through 1:64) of the serum samples (6). The Standard Tube Agglutination Test (SAT) developed by Wright and colleagues remains the most popular and easy test to perform. SAT can measure the total quantity of the agglutinating antibodies (IgG and IgM). The quantity of specific IgG is determined by treatment of the serum with 0.005M 2 mercaptoethanol (2ME), which inactivates the agglutinability of the IgM. However, many patients have low levels of agglutinating IgG antibodies and the results can easily be misinterpreted. SAT titers above 1:160 are considered diagnostic in conjunction with a compatible clinical presentation, however, in endemic areas the titer of 1:320 is taken as the cut off. Coomb's test is the most suitable and sensitive test for confirmation in relapsing patients with persisting disease, but it is complex and demands technique. Enzyme linked immunosorbent assay (ELISA) has become increasingly popular, as well as a standardized assay for brucellosis. It measures IgG, IgM, and IgA, which allows a better interpretation of the clinical situation. The specificity of ELISA, however, seems to be less than the agglutination tests. As the diagnosis of *Brucella* is based on the detection of antibodies against smooth LPS, the cut-off value needs to be adjusted, to optimize the specificity when used in endemic areas (7). ELISA can also be applied in the diagnosis of CNS brucellosis with varying success and further research must be aimed at improving the diagnosis of this condition. The Fluorescence polarization assay (FPA) offers a valuable alternative to conventional serological tests. This assay measures the size of a florescent tagged molecule such as an antigen — ideally antigens selected for this technique should be small (20 Kda). The utilization of the O-side chain of LPS from *Brucella* spp has shown encouraging results.The sensitivity of this test at the selected cut-off value is 96% for culture-confirmed brucellosis and the specificity is 98%.Immunochromatographic *Brucella* IgM / IgG lateral flow assay (LFA), a simplified version of ELISA has a great potential as a rapid point-of-care assay. Studies have shown that this test has high sensitivity and specificity for *Brucella* IgM and IgG. This system uses a drop of blood obtained by a finger prick, which is used by the bedside and easy to interpret. It is a rapid and simple diagnostic test for confirmation of brucellosis in an endemic area (7). In recent years new immunocapture agglutination for anti-*Brucella*(*Brucella* Capt BCAP) has been developed, to

detect agglutinating and non-agglutinating antibodies with high sensitivity. It has been suggested as a possible substitute for Coombs test and a better marker for disease activity (8). The main objective of this study was to determine the seroprevalence and exposure factors associated with human brucellosis in Albania so as to provide baseline information as well as give first indications about the extent of the problem in the country.

2. Material and Methods

This is a crossectionalsero prevalence study including specimens referred to the Institute of Public health in Tirana, Albania from patients suspected for Brucellosis over the period 2004-2012.All study participants were interviewed using a questionnaire which included demographics, risk factors and clinical symptoms for brucellosis. Sera were tested with the RBT for detection of antibodies to *Brucellaabortus/melitensis.* The ELISA test was performed according to manufacturer's instruction.
Statistical analysis

3. Results and Discussion

4020 patients were included in the study. The mean age of patients was 40.2 (±12.4) years with a range 1-84 years. 71.5% of study participants were males and 28.5% females. 43.5% of the study participants were farmers. The seroprevalence of *Brucella*spp. among participants was 19% (95% CI: 17.8–20.2) (fig. 1).Seroprevalence was higher in females than in males (11.2% compared with 10.9%, P = 0.029). By age group, the highest seroprevalence was found in those 15-24 years at 26.9% (95% CI: 13.9–17.0), with the lowest in the 1-4 year age group at 4.5% (95% CI: 1.5–20.4) (fig. 2).25% of rural residents tested positive for Brucellossis compared to 6.5% of urban residents (OR=4.7 95%CI 3.7-6.1), p<0.01. All occupation categories included seropositive cases. 346 (8.6%) of patients reported that they had a member of family with brucelossis. Reported clinical symptoms at the time of the study were compared to the sero-status of participants.
Overall, 63.4% brucellosis seropositive participants and 38.8% seronegative participants reported symptoms. Among all seropositives, 41.2% reported more than three symptoms; among the seronegatives, 18.2% reported more than three symptoms (P < 0.001). Headache; joint, back and muscle pain; night sweats and sleeping disturbances were significantly associated with brucellosis seropositivity. The higher seropositivity rate is observed during spring and summer seasons.

4. Conclusion

The findings of our study are similar with many other studies reported in literature (9-12).The prevention of brucellosis can be achieved through various measures. The most important step in preventing brucellosis in humans begins with the control and/or eradication of the infection in animals who serve as a reservoir. This requires a coordinated effort between local public-health organizations and animal-disease-control entities. The most effective measures to achieve this objective include animal vaccination programs, animal testing, and the elimination of infected animals (13). There is no human vaccine currently available. In addition to efforts to eradicate the disease in animals, preventive measures are aimed at reducing the risk of transmission to humans. If clinicians suspect brucellosis, the laboratory should be notified so that it can take the maximal precautions available. *Brucella* should only be manipulated by trained laboratory personnel in biosafety level II cabinets (ie, HEPA filters on both incoming and outgoing air), ideally in a BSL-3 level laboratory. Furthermore, additional workup of *Brucella*(eg, strain typing or antimicrobial sensitivities) should probably only be done in public health laboratories accustomed to working with aerosolized pathogens, and cultures should be kept sealed when not in active use. In the event of an accidental exposure, it seems reasonable to screen all workers in the involved area for the presence of agglutinating antibodies and to treat presumptively all those who are positive, as treatment of latent disease may result in a milder course.

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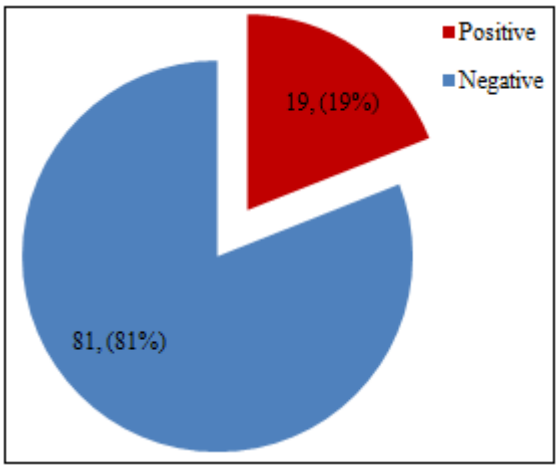

Figure 1: Seroprevalence of brucellosis

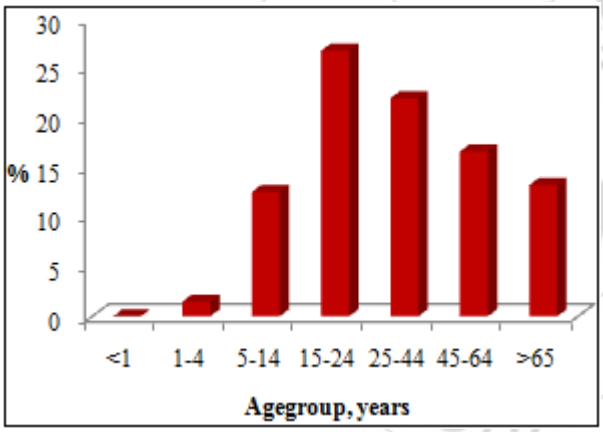

Figure 2: Seroprevalence of brucellosis by age group