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# BRUCELLOSIS IN SHEEP AND GOATS IN KOSOVO

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**Abstract**

*Sheep and goat Brucellosis in Kosovo has been reported in the last years and human brucellosis infections have mostly been in association with sheep and goat brucellosis. The aim of this study was the determination of the brucellosis immune status in three sheep herds with a small number of goats from two endemic regions in Kosovo due to high abortion rates in these herds. The possible association of sheep and goat brucellosis with human brucellosis cases from these households was aimed. The disease is endemic in Kosovo and surrounding countries. In the first herd out of 49 sheep and goats, 11 animals resulted positive using Rose Bengal Test (RBT) and Complement Fixation Test (CFT). 12 corresponding milk samples resulted positive using PCR and only one milk sample (goat) using horizontal method for isolation was confirmed as Brucella melitensis. In the second herd all of the 4 examined aborting sheep resulted brucella positive using both RBT and CFT. Out of 240 sheep in the third herd, 48 sheep resulted positive for brucellosis using RBT and 94 sheep resulted positive using CFT. This study shows the endemicity of brucellosis in Kosovo, the probably unknown brucella status of animals and the low vaccination efficacy.*

**Key words**: animal and human brucellosis, endemic, B. *melitensis*

## INTRODUCTION

Brucellosis is a global zoonosis and poses a severe public health challenge and socio-economic problems in several countries in the world. It counts as one of the major zoonotic pathogens worldwide and it is responsible for very big economic losses as well as high number of infected patients in endemic areas. The majority of cases occur in the Mediterranean countries of Europe and Africa, Middle East, India, Central Asia, Mexico and Central and South America. Four Balkan countries are considered as the most endemic countries and the region of Kosovo is not counted, where the burden of the disease has yet to be adequately quantified (Pappas, 2010). Regarding the former Yugoslavian republics a high prevalence in animals and humans was registered in the last years. Bosnia and Herzegovina counted 1639 human cases between 2011 and 2008 (Obradoviç and Veliç, 2010): in Croatia in 2008 Brucellosis was found in 370 sheep and goats and 3 cows using serology (Spicic et. al., 2010): in Serbia a prevalence of 26,8 % in sheep and 35 % in goats in 2004 was registered (Djuricic, 2006): in Bulgaria 105 cases and 635 animal cases were registered between 2005 and 2007 (Russo et al., 2010): in Macedonia 550 patients have been treated at the University clinic Skopje- Macedonia between 1998-2007 (Bosilkovski et al., 2010). The disease has been reported in the last ten years in Kosovo continuously in animals with high abortion rates and humans. In a study carried out in 7941 cattle's, 3548 sheep and 511 goats in Kosovo and the prevalence resulted 0, 58 % for cattle, 6,26 % for sheep, 7,24 % for goats (Jackson et.al., 2004). In average 30

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human cases with chronic brucellosis have been delivered in the Clinic for Infectious diseases in the last ten years (University Clinic for Infectious diseases-Kosovo, personal communication 2012), this doesn't include other cases treated in the regional hospitals and might count up to ten fold higher. In endemic areas the disease is often under-diagnosed and under-reported (Pappas et al., 2006). Although the routes of transmission have been known for decades and campaigns for eradication have been attempted in many countries and the consistently high numbers of cases indicates the challenges associated with preventing the transmission of this pathogen (Akhvlediani et. al., 2010). Some authors suggest that presently cross infections have been reported among food animals. Risk factors for humans are associated with consumption of contaminated milk and dairy products from sheep and goats and working with livestock in endemic areas (Lopes et al., 2010, Earhart et. al., 2009, Corbel 2006) direct contact with infected animal parts with open skin wounds or mucous membranes, and through the inhalation of infected aerosolized particles. An increased risk of exposition to brucellosis, the disability of the host"s defenses, the emergence of bacterial strains resistant to antibiotics and their widespread distribution as well its complexity associated with the action or inaction of man have been identified as the main factors leading to the emergence or re emergence of bacterial zoonoses (Blancou et al., 2005, Ruso et. al., 2009). In October 2011 a member of a household, a 16 years old boy after 2 weeks pain in his left leg, sweating and headache visited his ambulatory care physician and after examination he was instructed to the university orthopedic clinic and was hospitalized for 3 days. After further examinations he was transferred to the university clinic for infectious diseases. Brucellosis was confirmed using Wright test (1:1280) (Hamidi et al., 2012, unpublished data).

## MATERIAL AND METHODS

Three herds consisting mainly of sheep were involved in this study. All sheep belonged to the local race DKS (Delja e Kosoves) a common sheep race in Kosovo and were of different age. All the goats belonged to the Alpina Race. The animals are held in pasture throughout the year in all the farms. 240 animals mainly sheep from the first herd, 4 sheep from the second and 49 sheep from the third herd were tested serologically after suspicions for brucellosis due to the high abortion rates ranging from 40-45 % in the first and third herd and all animals in the second herd. The first and second herds were located in the southwest Kosovo, the third herd in the southeast Kosovo. Blood samples were taken out from all of the three herds. Additionally 47 milk corresponding samples were taken for serological and molecular analysis from the third herd. RBT was performed in all samples. Positive samples were further confirmed using Complement Fixation test (CFT) according to the International Standard Methods (World Organization for Animal Health, 2009). 14 milk corresponding samples (13 sheep and 1 goat) from the first herd were tested by PCR targeting the bscp31 gene (Baily et al., 1992). PCR positive samples were cultured and typed by classical microbiological methods (Alton et al., 1988) and semi-automated biotyping system (Al-Dahouk et. al., 2010).

## RESULTS

Out of 240 samples from the second herd, 48 samples (20 %) resulted positive in RBT and CFT, 46 samples were negative using RBT but positive using CFT (40 %). RBT and CFT results didn't match to each other. In The second herd out of 4 analyzed samples all animals resulted to be positive. Out of 49 sheep from the third herd using RBT and CBR, 11 samples revealed positive CFT titers (22,5 %) ranging between 1:20 (++++) and 1:160 (++++) . Results of both methods matched with each other. PCR revealed 12 brucella positive results. All the 11 samples which resulted positive using PCR were analyzed using the horizontal method for isolation of Brucella spp. and in only one revealed Brucella spp. colonies.

## DISCUSSION

The results presented in this paper show that the prevalence of brucellosis is high in sheep in Kosovo despite their vaccination. The high prevalence of brucellosis in investigated herds is associated with the pastoral practices and smallholder systems as a result of different management practices, animal handling and contacts and poor control of animal movement. All sheep herds involved in this study were vaccinated with a conjuctival dose for Brucellosis in 2010 using the vaccine named Brucevac (REV 1). Sheep and goat herds seemed to have been vaccinated without knowing the immune status of the herds. In fall 2010, the same year of vaccination a high number of abortions up to 40 % was registered in the first herd, 45 % in the second herd and in all 4 sheep from the third herd involved in this study. In 2011 no vaccination was performed and the abortion rate remained high. This study shows the importance of monitoring program for Brucellosis in endemic regions and determination of the immune status of sheep and goats. 48 animals in the first herd were positive using both RBT and CFT but another 46 samples were negative using RBT but positive using CFT, proving that RBT has a low sensitivity and specificity. This confirms findings by Blascou et. al 1994, whereas the specificity and sensitivity of CFT is higher in diagnosis of sheep and goat brucellosis. Human infections were registered from the second and third herd (Hamidi, et. al., unpublished data). The infection route seems to be through animal-human contact and cheese consumption which is produced traditionally without pasteurisation. It should be aimed to identify the burden of brucellosis responsible for high persistent animal and human prevalence. Therefore, to better understand the epidemiology of Brucellosis subtyping of Brucella strains is very essential (Al Dhouk et al., 2010, 2007). MLVA -16 method will allow a better understanding of the epidemiology of Brucellosis (Gwida et al., 2012). A study in the future has to be designed to address all related factors which contribute to the persistent high animal and human prevalence of brucellosis in Kosovo.

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