

INCIDENCE OF BRUCELLA MELITENSIS IN SHEEP LYMPHONODES

P.Sekulovski, M.Danev

Veterinary Institute Skopje Macedonia
ul. Lazar Pop-Trajkov 5, 1000 Skopje, Republic of Macedonia

Background

In Macedonia like in most South European countries' brucellosis is adopted zoonosis, mostly in sheep and goats being transmitted to human. Main sources of human infection are sheep milk and cheese. Little attentions have been paid to the role of sheep meat in the transmission of brucellosis.

An attempt was made to study the prevalence of brucellosis in sheep lymph nodes to determine the risk level of consumption of such a meat.

Material and Methods

Lymph nodes samples were collected from 50 sheep carcasses. Sheep were declared suspicious for brucellosis by serological methods and consequently slaughtered in a sanitary slaughter house for the purpose of this study. Three lymph nodes were taken from every sheep: Lnn.supramamarii, Inn. iliaci medii and Inn. inguinales superficiales. These were chilled on ice bricks and immediately forwarded to the laboratory and frozen at -20°C for 2 weeks.

Samples were dipped in ethyl alcohol and flamed to remove the external contamination. Each sample was cut into small pieces and homogenized with equal volume of phosphate buffered saline in Stomacher 400 (Seward Medical-UK). One ml of homogenate was transferred to two tubes of Brucella Selective Broth (Oxoid Tryptone Broth, CM129 with Oxoid Antibiotic Supplement, SR83) and incubated for 7 days aerobically and anaerobically at 37°C. Solid media were inoculated with swab-sticks directly from the macerate and at 4th and 7th day from the enrichment. Isolation and identification were done accordingly to Alton and Jones (Techniques for the Brucellosis Laboratory, INRA) with slightly modification of the culture mediums used. Glycerol-dextrose agar, TSAEV (tryptose agar with serum, antibiotics and ethyl violet) and Farrell medium were used for plating.

Isolates were tested and typed for the following characteristics: sensitivity for dyes (thionin and basic fuchsin 1:50000, 1:100000), urease activity, oxidase activity, catalase activity, acriflavine test, agglutination with monospecific sera (anti-A, anti-M and anti-R) and ability of phages to lyse culture (Tb, Wb, Iz, R/C).

Results

Out of 150 samples (from 50 sheep carcasses) examined culturally, 9 (from 7 carcasses) revealed the presence of brucella organisms, giving the prevalence rate of brucella infection in sheep as 14 %. Brucellae were isolated from 6 lnn. supramamarii out of 50 (4%) and 3 lnn. Illiaci medii out of 50, too (2%). In two cases the same sheep harbored Brucellae in both lnn. supramamarii and lnn. illiaci medii.

Best results were obtained from the plates streaked at 4th day. Prolonged incubation for 7th days did not dramatically improve the isolation rate. Enrichment in selective broth proved to be very effective for isolation of Brucellae from tissue samples. Farrell agar turned out to be the most susceptible solid medium for the isolation of Brucella species.

On the basis of biochemical and serological tests the isolates were confirmed as Brucella melitensis, biotype 2.

Discussion

From 1980 Brucellosis is significant epidemiological problem in Republic of Macedonia like a result from epizooty among sheep and goats. Up to date studies point on milk and milk products and work with infected animals as two main sources of human infection. Nothing or very few is published about sheep meat as a potential risk for man

Our study revealed that 14 % of sheep (7 from 50) were found positive in bacteriological examinations of their lymph nodes. Nine strains of brucella melitensis biotype 2 were isolated from supramammary and iliac lymph nodes. Two sheep contain brucellae in more than one group of lymph nodes.

Enrichment in selective broth with addition of antibiotics for 4 days and plating on Farrell Selective medium performed best results for isolation of brucellae from lymph nodes.

Isolation of Brucella microorganisms from sheep tissues, in Macedonia, has been reported probably for the first time.

The present study confirmed our prediction that sheep lymph nodes could be a serious threat for human health as a part of the meat. Mostly the raw or fresh meat can possibly cause such an infection but our study revealed that also frozen meat can be dangerous for human health if contains brucellae.

Considering the above mentioned we suggests thoroughly cooking before meal and , regarding the meat inspection, condemnation of iliacs and supra mammary lymph nodes.

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