

ABSTRACT

Thesis entitled **„BACTERIOLOGICAL, EPIDEMIOLOGICAL AND SEROLOGICAL RESEARCHES IN BRUCELLOSIS OVINE“** is scientific and practical reasons the following:

- Infectious epididymitis in Romania, described since 1957, continues to be one of the diseases commonly found in animals, causing major economic losses through depreciation rams sheep breeders, abortions and births of dead or viable in sheep products as well as the costs of surveillance measures, prevention and control, with infection as a result decreasing share of fecundity;
- Although as a consequence of animal health measures to prevent and combat infectious epididymitis was considerably reduced, *Brucella ovis* infection can be controlled with maximum efficiency due to ignorance of the actual incidence among susceptible animals;
- Although surveillance program, prevention and control of animal diseases requires systematic serological surveys, found persistence of outbreaks of infectious epididymitis in territory considered;
- Were found failures in eradicating infection with *Brucella ovis* largely due to insufficient sensitivity and specificity of various screening and diagnostic tests, inconsistency of measures of prevention and control and not least, existence of poorly known sources of infection may contribute to the spread of disease.
- Present work has as objective to highlight issues relating to methods of diagnosis and epidemiology of the disease.

The thesis have 161 pages and is structured in accordance with existing legal provisions, into two main parts: the first part entitled **“State of knowdlege”** include 45 pages representing 28 % of sentence and the second **“Researches personal”** representing 55,3 % of thesis. The work includes 23 tables and 67 figures (graphs, maps and photos), for a better presentation of content. References consulted include 165 securities of domestic and foreign literature.

The first part - “*State of knowledge*” three chapters, are presented briefly, information from literature on the topic sentence are presented briefly, information from literature on the topic sentence, data were used to interpret and compare results obtained in the second.

The first chapter presents data on the history and taxonomy of the genus *Brucella* and morphological characters, cultural, biochemical and pathogenicity of brucellosis.

Chapter II “Implications of pathogenic species *Brucella ovis*” is described based on the literature consulted, *Brucella ovis* infection. Accelerated method of transmission of the species *Brucella ovis* in sheep. It showed morphological and cultural characters of the species *Brucella ovis*, the epidemiology, pathogenesis, clinical and pathology, prevention and control of infection with *Brucella ovis*.

Chapter III contains procedures for diagnosis of infectious epididymitis in bacterioscopic and bacteriological examination and serological diagnosis by complement fixation and ELISA immunoenzymatic test.

Part II “*Researches personal*” contains four chapters, the general conclusions and bibliography are presented and discussed results of investigations conducted.

In Chapter IV are presented epidemiological research on *Brucella ovis* infection in Vaslui. Epidemiological investigations were conducted in 2000-2008. The study was conducted on livestock breeding rams in the county.

Were processed and utilized a multi-series data on the evolution of infectious epididymitis in Vaslui. Data processing was carried out by various mathematical methods, from simple arithmetic average of multi-strings to formulas established in the assessment of intrinsic value allergic test (sensitivity, specificity) and the establishment of epidemiological indicators: prevalence and incidence.

Thus, in Vaslui county, in the period analyzed, the maximum disease incidence was 155 cases (2.84 %) in 2002, with a minimum of two cases (0.03 %) in 2005. Annual prevalence of infection with *Brucella ovis* ranged from a value of 6 (0.10 %) in 2005 to a value of 157 (2.88 %) in 2002. Highest incidence of cases was detected serologically in 2002 (155 cases) and 2007 (137 cases), multi-media cases of brucellosis in rams being 82,66. Were detected in 2007 in 22 locations (53.66 %) 137 cases of seropositive infected rams to RFC, municipalities located in the north-east of Vaslui County.

Chapter V, entitled ” Research on the significance of bacteriological and serological tests in diagnosis of infection with *Brucella ovis*” include research findings on the significance of

bacteriological tests in the diagnosis bacterioscopic and *Brucella ovis* infection, serological investigations represented by the complement fixation and ELISA immunoenzymatic test and correlation of their results.

In subsection 5.1. are the results obtained from examinations conducted bacterioscopic of organs and tissue sections from each area were found congestion, infiltration, caseous, densification by pressing blades fingerprints.

Between 2000-2008, there were 234 samples examined bacterioscopic. Cocobacilare bacteria were detected in a total of 145 samples (61.97 %).

Dried preparations were then fixed and stained by dye techniques: Stamp method (Ziehl-Neelsen modified) recommended by the O.I.E. and O.M.S. Direct microscopy performed correctly identified by the said stains, bacteria cocobacili or small, intense red color, a pale green background, respectively, blue, isolated or in groups diplomacy, often in small piles, with intracellular localization, especially polymorphonuclear leukocytes.

Growing brucellelis stressed in subsection 5.1. – “*Bacteriological exam*”. Cultural examination was the preparation of pathological material and culture media, their seeding, followed by reading their incubation and cultures bacterioscopic.

Cultural examination was performed on samples taken from ram testes from 234 seropositive cases.

Primary or secondary crops produced on solid or liquid media were examined to identify characteristics that may lead to growth of *Brucella* species typing.

In liquid culture media, *Brucella ovis* produces a low turbidity and moderate, consistent with powdery deposit on the surface and possibly ring.

On solid media after 48-72 hours, colonies appeared rough, type R, with dry matter, granular, golden-yellow color, with varying sizes (2-4 mm diameter) at 36-38°C temperature at a pH of about 7 and CO₂ in the atmosphere (fig. 5.7-5.12.).

The species *Brucella ovis*, due to antigens M (melitensis) and A (abortus), not observed cultural type S.

In subsection 5.2 presents the results obtained by serological examination by complement fixation. The study was conducted on livestock breeding rams in the county.

In the period 2000-2008 were examined serum samples and 103 194 have identified a number of 744 (0.72 %) serologically positive rams, while 125 samples (0.12 %) were resulte by anticomplementary.

Anticomplementary samples were reviewed by another inspection at 21 days, 12 (9.6 %) of them have achieved positive results and 21 samples (16.8 %) remained anticomplementary nature.

Animals slaughtered (234) for the diagnosis of brucellosis in rams (31.45 %) samples were sent to the national reference laboratory (testes and epididymis). After bacteriological examination were 112 confirmed cases (47.86 %).

Also, the RFC was obtained a total of 116 (0.11 %) samples with doubtful side (the + +).

Immunoenzymatic ELISA assay were all examined blood samples that were positive and dubious RFC and other blood samples from breeding rams.

In 2006 was obtained from examination RFC hit the 125 samples and 59 samples from a number of results were anticomplementary. Following these results, ELISA was performed on positive samples. Results obtained from the test ELISA positive animals have reduced the number of samples in RFC 125 80 samples (64.00 %) while the dubious results were obtained from 24 samples (19.02 %), the remaining 21 samples (16.80 %) negative.

In 2007 were examined by immunoenzymatic test ELISA positive samples in RFC 137. ELISA gave a positive result from a number of 125 samples (91.24 %), 12 samples (8.76 %) is questionable.

Immunoenzymatic ELISA test was performed in 2008 on 48 serum samples from serologically positive rams RFC. Following test were obtained 33 (68.75 %) positive samples, 8 (16.66 %) dubious evidence, the remainder being negative.

Of the 310 serum samples with positive breeding rams to RFC, immunoenzymatic test was positive in 238 samples (76.77 %), suspicious in 44 samples (14.19 %) and negative in 28 samples (9.03 %).

Another aspect included in the study is the correlation diagnostic tests with *Brucella ovis* infection.

In the period 2000-2008 were examined serologically for infection with *Brucella ovis* - 103 194 serum samples from breeding rams, 744 (0.72 %) of them being positive. A total of 310 samples from seropositive rams were worked by immunoenzymatic ELISA test, 238 (76.77 %) were positive in this test.

From a total of 234 rams were seropositive to RFC harvested testes and epididymis after castration or slaughter for laboratory tests: anatomical and bacterioscopic (conducted in laboratory county) and germ (the national reference laboratory).

In the study we used samples from breeding rams from infected flocks and herds free.

Following serological tests were seropositive rams slaughtered 234, suspected of infectious epididymitis, to confirm the diagnosis. Of these, 79 (33.76 %) had gross lesions characteristic of disease. After bacteriological examination, the disease was confirmed in 112 samples (47.86 %).

Pathological examination in 468 pairs of testicles orhiepидidimitis showed lesions in 18 pairs (3.85%). These lesions were represented by: abscess in the head or tail of the epididymis, microabcese parenchymal, calcification in the parenchyma, testicular hypertrophy or atrophy in the epididymis.

Making the correlation between the presence of specific pathological lesions and epididymitis bacteriological confirmations were obtained the following results: 18 samples was isolated lesions *Brucella ovis* in pure culture for 12 samples (66.67 %); *Brucella ovis* associated with *Arcanobacterium pyogenes* (*C. pyogenes*) in three samples (16.67 %), *A. pyogenes* in a sample (5.55 %) and two samples (11.11 %), bacteriological examination was negative.

Bacteriological examinations performed for confirmation of infection with *Brucella ovis* was performed on a total of 234 samples testes and epididymis. The result of this examination showed that orhiepидidimitis was caused by the following types of bacteria:

- *Brucella ovis* : 47,86 %;
- *Brucella ovis* associated with *Arcanobacterium pyogenes* (*C. pyogenes*): 6, 42 %;
- *Arcanobacterium pyogenes* : (7,69 %).
- Other bacteria species: (*Staphylococcus* spp., *Streptococcus* spp., *Escherichia coli*, *Pseudomonas aeruginosa*, *Candida* spp.): 10,68 %.

Given that RFC is a standard method for diagnosis of *Brucella ovis* infection and comparing the results of bacteriological examination on the same samples were diagnosed positive RFC was found between these two methods is a positive correlation.

Correlation of necropsy and clinical examination, on the one hand, and serological tests were negative: only 4.17% of rams were diagnosed serologically positive specific epididymal lesions and clinical changes.

To determine the value of screening tests used in diagnosis of infection with *Brucella ovis* was determined their specificity and sensitivity.

Thus, complement fixation reaction (CFR) is a sensitive serological method of diagnosis (67.07 %) but poorly specific (48.09 %) due to *Brucella ovis* antigenic interference with other germs.

Study showed that ELISA has better sensitivity (80.00 %) than the RFC (67.07 %).

Due to a number of 28 serum negative in ELISA and positive in RFC, the combination of these two tests could give a much higher sensitivity.

To avoid false positive reactions to rams of biological and economic value, is required at least two different methods diagnostic (RFC or RFC and ELISA and bacteriological examination and semen).

To ensure indemnity of sheep against infectious orchitis be respected national strategic program specific elimination by slaughter of breeding rams serologically positive.

Most effective tool for serological diagnosis of brucellosis can only be a combination of the two tests, but no one test case from those presented in the paper.

Chapter VI, entitled “Epidemiological and clinical research in infections with *Brucella ovis*” includes study in seropositive infected rams to RFC in 2000-2008. Following clinical examination, were found in 348 (46.78 %) seropositive rams orchitis clinical changes occurred, which led to suspicion of infection with *Brucella ovis* and rams at 396 (53.22 %) were not detect changes in clinical examination.

Demonstrated that there is a negative correlation between clinical signs and serological examination results.

This study demonstrates the importance of annual serological surveillance tests rams before and after the mating. In the county, *Brucella ovis* infection was widespread and occurred in the mating season rams and in its immediate aftermath.

During 2000-2008, the flock of sheep in the county had a generally upward trend, reaching from 222.475 in 2000 to 272.424 in 2008. In this context, increased the number of breeding rams, from 4836 to 2000 to 6382 in 2008, with a maximum of 6700 in 2007, when in fact been detected by clinical examination and 117 rams sacrificed to confirm diagnosis *Brucella ovis* infection.

Serological surveillance examinations in breeding rams were performed 2 times per year, from september to november, before and after mating.

Regarding the clinical examination, it was made by observation and palpation. Were examined serologically positive rams which reacted to RFC and those whose infection was confirmed and were directed to slaughter. 234 rams were slaughtered, from which pairs were sampled which were the testes and epididymis. Some of these samples have been working on the county laboratory and some were sent to the national reference laboratory for confirmation. Following the results of bacteriological tests, infection was confirmed in 112 cases.

Were clinically investigated a number of 744 breeding rams with positive results in infectious epididymitis surveillance examination by RFC.

Regarding the strategy of surveillance and control of infection with *Brucella ovis* in Vaslui, one can say that, in terms of evolution of *Brucella ovis* infection, this is an important activity in order to maintain free sheep. Therefore, national central veterinary authority, the authority to approve national strategies, deliver annual veterinary rules on how they can get allowance sheep. According to annual surveillance and control strategy of *Brucella ovis* infection is achieved by including as a mandatory action, surveillance herds of rams, as provided in program oversight activities, prevention, control and eradication of animal diseases, those transmissible from animals to humans, animals and environmental protection, other actions provided for in other national programs.

The program requires that the sheep should not introduce new animals purchased, unless they come from herds free. Artificial insemination of sheep to be used only morphological and bacteriological semen controlled, derived from rams ensure free and negative in three complex examinations. Avoid contact with other herds, directly or through the paddock.

Serological monitoring is required by RFC, the breeding rams with two weeks before and after mating season. It is also mandatory serological control of brucella infection to rams, goats, sheep and goats breeding in cases of sale.

In our country do not practice active immunization of animals against brucella infection.

Confirmation of disease in complex laboratory tests lead to herd sanitation.

To this end, all animals with clinical signs of disease and positive serological actually be deleted from. Elimination of sick animals is followed by rigorous disinfection. Sewage is considered completed when two complex examinations (clinical and serological), quarterly positive cases are not recorded.