

CORRELATION STUDY BETWEEN POSITIVE SEROLOGIC TESTS AND BACTERIOLOGICAL AND MORPHOPATHOLOGICAL EXAMINATION FOR *BRUCELLA OVIS* INFECTION IN THE AREA OF CLUJ COUNTY

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Abstract: Research was conducted during 2000 - 2004 on a number of 60.986 blood samples from rammers; 530 pairs of testicles for bacteriological examination and 76 testicle samples processed for histopathology. Serologic and morphopathologic investigations were performed in the Veterinary State Laboratory Cluj (LSVS) and the bacteriology department in the Institute of Diagnosis and Animal Health (IDSA) Bucharest. Following the examination by serological RFC resulted a number of 4200 positive tests from 60.986 samples tested. Thus; the percentage of sickness caused by infectious epididimitis in rammers is 6.88%. Investigations by bacteriological examination; conducted at IDSA Bucharest on a number of 530 samples testicles (representing evidence and aries); revealed the presence of infectious epididimitis of rammers at 77.36%; 22.64% of the samples were negative. Having established a hierarchy of isolated germs involved in the aetiology of epididimitis we observed that 62.02% of cases are caused by *Brucella ovis*; 6.60% of cases by *B. ovis* in association with *Arcanobacterium pyogenes* and 7 ;74% by *Arcanobacterium pyogenes* only. Histological examination was conducted on a number of 76 testicles samples; of which 46 samples (60.52%) were with pathologic changes that can be attributed to infectious orchiepididimitis of rammers. Correlation is positive between histological examination and bacteriological examination; from a proportion of 100% testing positive for pathology; we managed to isolate *Brucella ovis* from 83.33%. Between necropsic exam results and serological examination by RFC; the correlation is negative; only 4.17% of the proportion of 100% diagnosed serological; presents pathological lesions of epididimitis. This type of relationship is valid also between serological examination by RFC and palpatory clinical examination. Considering RFC as a standard method to diagnose infectious epididimitis and comparing results with those of bacteriological examination carried out on the same samples that were diagnosed positive with RFC; we found that between these two methods there is a positive correlation. If RFC detected positive samples in the proportion of 100%; bacteriological examination detected the evidence of infected germs in a proportion of 77.36%; of which the *Brucella ovis* is 69.62%. Following bacteriological tests carried out on the same samples diagnosed as positive by serological examination (RFC); we find that the reaction of complement fixation although very sensitive; does not meet all the requirements; especially in regard of specificity; detecting also other bacterial antigens similar to *B. ovis*. For these reasons; in order to avoid false positive reactions from rammers with high economic and biologic value; testing is required to be made by at least two different methods (RFC and ELISA or RFC and bacteriological examination of semen).

INTRODUCTION

It is an enzootic infectious disease; with usually chronic evolution; which affects sheep; especially male; shown by fertility reduction; epididimitis or orchiepididimitis and inaparent in females; sometimes by abortion; birth of physical unstable lambs and temporary sterility (9). *Brucella ovis* infection continues to have a large spread; the disease has only economic interest and it's characterized by depreciation of rammers which can not be used for reproduction and decreased state of birth; which becomes evident in herds where the infection

rate exceeds 10% of rammers (6). Infectious epididimitis of rammers increases in frequency in the reproductive season and the period immediately followed.

Diagnosis in infectious orchiepididimitis of rammers is based on data obtained from clinical; bacteriological (*Brucellei ovis* isolation from semen) and serological (positive results in blood samples) examination (3). On the other hand; should be considered the epidemiological situation of the holding; related breeding; vaccination and history of orchiepididimitis. However diagnosis of an animal with epididimitis is only indicative; for the confirmation of the disease should be made a differential test. In our country the infectious orchiepididimitis or rammers produced by *B. ovis*; must be differentiated against infections with *Arcanobacterium pyogenes* (*Corynebacterium pyogenes*); which can evolve sometimes together and against infections with *Actinobacillus spp.* Positive differentiation in these cases is possible only through the bacteriological examination (5).

In case of confirmation of the disease; all animals with clinical signs and serological positive must be eliminated; a disinfection must be made and other livestock must be examined from the clinical and serological point of view and the resut decides their situation (7).

The dynamics of disease outbreak in epididimitis is characterized as enzootic with limited extension; slow expansion; higher at reproductive season and the period immediately followed (2). The contribution of laboratory testing is indispensable for the confirmation of the disease.

The negative result of the bacteriological examination does not exclude the presence of *B. ovis*; given the nature of chronic disease in which germs are eliminated intermittently or not eliminated at all.

MATERIAL AND METHODS

The aim of this project is to present the methodology used for the diagnostic in infectious orchiepididimitis of rammers in Cluj county; where endemic disease progresses; the correlation between the methods and possible diagnostic errors.

As sources of information we used statistical data accumulated over a period of 5 years; 2000 - 2004; at the Directorate of Veterinary Health and Food Safety Cluj.

Data collection was carried out from the archive of Veterinary State Laboratory Cluj; according to classic epidemiological methods.

The research was conducted in the Veterinary State Laboratory Cluj (LSVS) and the Institute of Diagnosis and Animal Health (IDSA) Bucharest at the profiles: Serology; Bacteriology – Pathologic Anatomy; Histology LSVS from Cluj and in Bucharest at Bacteriology - IDSA during 2000 - 2004; at a number of 60.986 blood samples from rammers; 530 pairs of testicles for bacteriological examination and 76 testicle samples histological processed.

Serological examination for infectious orchiepididimitis of rammers; in state veterinary laboratories (LSVS) is currently made only by the complement fixation reaction (RFC) (1); working technique approved in the Institute of Diagnosis and Animal Health Bucharest (4). Testicles harvested after castration or slaughter of the rammers; serological positive; were sent to LSVS Cluj for laboratory examination. Laboratory investigations consisted of: necropsy; pathology and bacteriological exams;. Necropsy examination was performed only at one of testicle; the second was sent to Bucharest IDSA for bacteriological examination.

The conduct of the laboratory diagnostic consisted on tracking the following stages: the collection of samples; serological examination; necropsic examination; bacteriological examination; histological examination; the correlation between the results of serological and the bacteriological examination; correlation between the result of bacteriological and histological examination; the correlation between necropsic and bacteriological results.

It was tracked the correlation between the rate of detection at the complement fixation reaction (RFC) and indirectly the incidence of infectious orchiepididimitis of rammers in Cluj.

At the necropsic exam there were monitored specific anatomopathologic lesions of infectious epididimitis; which can still be highlighted during the life of the animal through a simple clinical examination and determination of correlations between the presence of them and serological; bacteriological and histopathological confirmation of the infection with *Brucella ovis*.

By bacteriological examination we aimed to establish the incidence of epididimitis caused by *Brucella ovis*; *B. ovis* associated with other bacterian agents and epididimitis caused by other microorganisms.

In the period under study (2000 - 2004); the price of the cost for diagnostic tests performed for rammers epididimitis has been supported from the state budget.

RESULTS AND DISCUSIONS

Following the examination by serological RFC; it has been established that in county Cluj during 2000-2004 the percentage of sickness caused by infectious epididimitis of rammers is at 6.88%; 4200 of rammers positive of 60986 tested.

Serological investigation result; the number of positive tests and the percentage for the incidence of infection in the herd of rammers examined and the structure of ownership is presented below.

Table no. 1.

The result by serological RFC examination for the diagnosis of infectious orhiepididimitis of rammers in Cluj county; in the period 2000-2004.

Year	No. of samples	Positive	
		No.	%
2000	10202	764	7;48
2001	12772	272	2;12
2002	10871	2118	19;48
2003	12848	642	4;99
2004	14293	404	2;82
TOTAL	60986	4200	6;88

Necropsic examination on 432 pairs of testicole rammers has shown injuries that pleede for *Brucella ovis* infection for 18 pairs; representing 4.17%. These lesions were represented by: abcesses at the head or tail of epididimus; microabcesses in testicle parenchim; calcifications in parenchim; epididimus or testicular hypertrophy; or testicular periepididimitis atrophy.

Following the establishment of a correlation between the presence of specific anatomopathologic lesions for epididimitis and positive bacteriological examination at the

evidence in question; there were obtained the following results: from 18 samples with lesions it was isolated *B. ovis* in pure culture from 12 samples (66.67%); *B. ovis* associated with *Arcanobacterium pyogenes* (*C. pyogenes*) of 3 samples (16.67%); *A. pyogenes* to a sample (5.55%) and at 2 samples (11.11%) bacteriological examination was negative.

Bacteriological examination to confirm *Brucella ovis* infection for rammers was made at the IDSA Bucharest on a number of 530 testicle samples; of which 360 samples were shipped in 2002 and 170 in 2003. The result of this examination reveals that infectious orchiepididimitis of rammers in Cluj is caused by the following bacterial types: *Brucella* (63.02%); *brucella* associated with *Arcanobacterium pyogenes* (*C. pyogenes*) in a proportion of 6.60% and only *A. pyogenes* in a proportion of 7.74% as may be established and the figure 1.

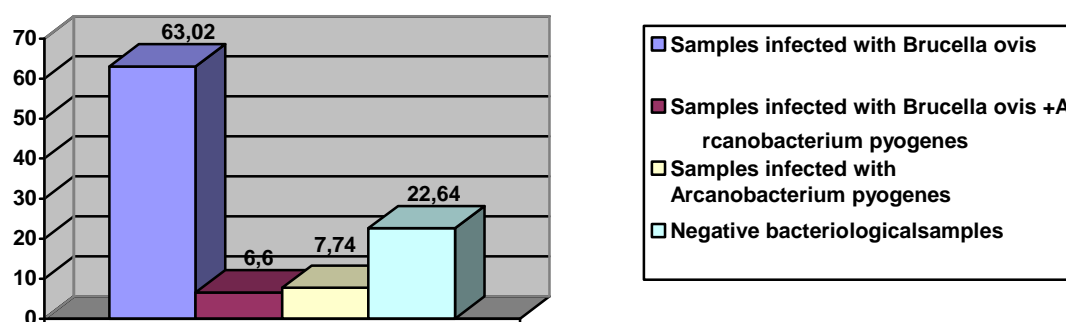


Fig. 1. The incidence of various types in the aetiology of infectious orchiepididimitis of rammers in Cluj; in the period 2000-2004.

Histological examination was made on a number of the 76 testicle samples of which 46 samples (60.53%) were detected with pathologic changes that can be attributed to infectious orchiepididimitis of rammers. The most common changes regarding histopathology encountered in histological sections were: epithelium degeneration of epididymary canalicules and spermatic stase; limpho-histiocitary infiltration in intertubular interstice; spermatic granuloama at the tubular level with polymorphonuclear infiltrate necrotico-purulent epididimitis in the outbreak; limfo-histiocitary epididimitis; granuloama with central coagulation necrosis; giant-epiteloides reaction and peripheral limphocitar infiltrate.

Considering that method RFC is the standard diagnostic for infectious orchiepididimitis and comparing the results with those of bacteriological examination carried out on the same samples that were diagnosed positive with RFC; it was found that between these two methods there is a positive correlation. If RFC-positive samples detected in the proportion of 100%; bacteriological examination detected evidence of infection with germs in a proportion of 77.36%; of which the *Brucella ovis* proportion was 69.62%. Following these issues and those 18 pairs of testicles with specific anatomopathologic lesions of epididimitis; we found that the proportion of detection from bacteriological examination increase significantly. Correlation is also positive between histological examination and bacteriological examination; from a proportion of 100% testing positive for histopathology; it was managed to isolate *Brucella ovis* of 83.33%.

Between necropsic exam results and serological examination by RFC; the correlation is negative; only 4.17% of the proportion of 100% diagnosed serological; presents specific pathological lesions of epididimitis. This type of relationship is also valid between serological examination by RFC and palpatory clinical examination.

These data reveal the high value of the bacteriological examination as a method of diagnosis; although a proportion of 11.11 to 22.64% of the samples were negative; this result should not be charged to the method but rather to other causes (incorrect harvesting and storage of the samples; autosterilization of the animal with footprint immunological remanence; portage infection or other related antigenic agents; giving false positive reactions.

CONCLUSIONS

- The complement fixing reaction (RFC) is a serological diagnostic method sensitive but insufficiently specific because of antigenic interference of *B. ovis* with other germs rerealing false positive reactions.
- In order to avoid false positive reactions from rammers with high economic and biological value; testing is required to be made by at least two different methods (RFC and ELISA or RFC and bacteriological examination of semen).
- To ensure indemnity of the sheep herds against infectious orchipeididimitis; must be respected the national strategic programme; developed by ANSV; and eliminated from reproduction seroloical positive rammers.

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