ABSTRACT

Keywords: goats, caprine arthritis encephalitis, CAEV, AGID, nested PCR, sequencing

The fact that more and more farmers prefer breeding goats from specialized races for milk production and that most of our knowledge is derived from analogy with cattle or sheep pathology has led to the need to carry out research into the pathology of caprine arthrithis encephalitis.

The growth of goats, especially specialized breeds for milk production, is facing worldwide with an important infectious viral pathology responsible for the occurrence of diseases that cause significant economic losses to the breeders (eg. milk production decline, weight loss, lagging behind increase, decrease of immunity and predisposition to secondary infections and gastrointestinal parasitism, decrease of the period of exploitation). One of these disorders is infection with lentivirus of small ruminants, *caprine arthritis encephalitis virus* (CAEV).

The PhD Thesis titled "**Research on arthritis of caprine encephalitis in Eastern Romania"** was aiming at identifying the presence of infection with the *caprine arthrithis encephalitis virus* by different diagnostic methods as well as the elaboration of a strategic plan of control and surveillance as sustainable as possible.

The originality elements of this work are conferred by the clinical and anatomopathological study of the caprine arthrithis encephalitis on the goats for the first time in Romania, as well as the correlations and statistical differences of the results obtained during the serological tests and molecular biology.

The doctoral dissertation is written in 13 chapters, contains 200 pages and is structured according to the scientific criteria in two main parts: the first part, entitled **"Current state of knowledge"** and second part **" Personal Contributions "** to which is added the content, the abstract, the related bibliography and the annexes.

The first part, **"Actual stage of knowledge",** consists of 35 pages, representing 17,50%, and is structured in 4 chapters, which synthesizes the main bibliographic data, the etiological, epidemiological, clinical and anatomopathological, diagnostic, surveillance of the caprine arthrithis encephalitis on goats, with the latest updates in the field of literature based on 304 bibliographic titles.

The second part "**Personal contributions**" is structured in 9 chapters, it contains 155 pages, representing 77,50%, contains 33 tables, and 106 figures. Each chapter of the second part contains material and working methods, the results obtained with their discussion and the partial conclusions, the last chapter synthesizing the final conclusions of the researches obtained.

In the first chapter titled **"Bibliografic data about the history and etiology of caprine arthrithis encephalitis"**, data are presented regarding the history and classification of the etiological agent, as well as the spread and importance of this infection.

The second chapter entitled **"Epidemiology and pathogenesis of infection with *caprine arthrithis encephalitis virus (CAEV)*"** contains 5 subchapters, presenting scientific data on epidemiological characteristics, receptivity, sources of infection, ways of penetration of the virus in the organism, mode of contamination, epidemiological dynamics and pathogenetic mechanism.

In chapter III **"*Caprine arthrithis encephalitis* symptomatology and morphopathology"** were described the clinical, anatomopathological and histopathological changes caused by the *caprine arthrithis encephalitis virus*.

Chapter IV - **"Diagnosis, surveillance and control of caprine arthrithis encephalitis"** treats the main diagnostic methods, referring to the etiological and differential diagnosis, as well as the surveillance and control measures to be applied to limit the spread of the disease.

Chapter V entitled **"The organizational and institutional framework in which the research was carried out"** describes the institutions and organizational frameworks in which the research was carried out during the 4 years.

Chapter VI entitled **"The purpose and objectives of the research"** presents the purpose and the objectives proposed for this research.

In Chapter VII**, "Epidemiological observations regarding on infectious pathology of goats in the East of Romania"** are presented the epidemiological researches carried out during the 4 years with the purpose to highlight the presence of infectious pathology in the goat population in the eastern region of Romania, the correlation of the pathology with the breeds , the age category, the type of holding, the factors that contributed to the occurrence of the diseases as well as the prevention and control measures.

Within the epidemiological investigations carried out between October 2014 and June 2018, 8628 goats of different breeds and age categories were examined, as well as exploitation systems in the counties of Botosani, Iasi and Vaslui. As a result of the clinical examinations carried out in goats, clinical signs were found at 14,19%, respectvely 1224 goats. Infectious diseases were present in all breeds and age categories. The most significant of weight loose was recorded in specialized breeds of 64,22%, respectively in 786 animals, for adult ones it was 53,35%, respectively 643 animals, slightly higher than the young goat which registered a weight loose of 46,65%, respectively 571 goats. The most common diseases are those caused by infectious pathogens such as*: Caprine Artrithis Encephalitis Virus, Orf Virus, Pasteurella multocida, Escherichia coli, Mycoplasma agalactiae, Dichelobacter nodosus and Fusobacterium necrophom.*

In Chapter VIII - **"Research on seroprevalence of *caprine arthrithis encephalitis* in the East of Romania"**, the seroepidemiological researches carried out during the period 2014-2018 in goats from the three counties are presented, aiming at describing the epidemiological features and determining the seroprevalence of caprine arthrithis encephalitis both in goat farms as well as from households in the counties of Botosani, Iasi and Vaslui. From the analysis of the results obtained from the researches performed on the diagnostic significance of the serological tests performed for CAEV

antibodies, it was found out that of the 762 serum samples at 17,59% and 134 sera, respectively, positive reactions were obtained, and 82,41% and 628 sera, respectively, reacted negatively.

In Chapter IX **"Clinical and paraclinical investigations concerning the *caprine arthrithis encephalitis at* goats in the East of Romania"** there are 2 subchapters, the first subchapter describes clinical aspects of the disease in young goats and adults, and in the second subchapter are described aspects of imaging exams. Investigations, when CAEV infection was the suspected cause of clinical disease, aimed both at highlighting and correlating the clinical manifestations present in viral infection with the imaging aspects present in the elective organs. Infection with the *caprine arthrithis encephalitis virus* evolves in specialized goats breeds, chronically localized, with poor clinical signs in all age groups, with values ranging from 2,25% to 8,99%. In goat youth up to 6 months of age, there was a subacute clinical form with the progression of pneumonia with progressive weakening. Adult goats found a chronic evolution with articular, respiratory and mammary localizations, clinically expressed by degenerative chronic arthritis, interstitial pneumonia and induration mastitis.

Further, chapter X **"Research concerning morphopathological aspect in caprine arthrithis encephalitis at goats in the East of Romania"** describes the macroscopic and microscopic lesions of caprine arthrithis encephalitis which were highlighted by necropsy and histopathology examinations. Investigations when CAEV infection is the suspected cause of clinical disease were aimed both at highlighting and correlating post-mortem macroscopic and microscopic lesions following infection not only in the virus's chosen tissues.

Chapter XI **"Research concerning the confirmation of caprine arthrithis encephalitis diagnosis"** is structured in two subchapters, the first subchapter includes research on the confirmation of diagnosis of caprine arthrithis encephalitis, detection of CAEV antibodies by the AGID method, and the second subchapter includes research on confirmation of diagnosis of caprine arthrithis encephalitis, detection of nucleic acid by the nested PCR method. Following research on confirmation of the diagnosis of caprine arthrithis encephalitis, the detection of antibodies by the AGID method carried out in 2014-2018 in the three counties to 762 serum samples from goats from the three counties showed a positive response to 17,59% respectively, to a number of 134 goats and negative response to 82,41%, respectively to 628 goats. Following research to confirm the diagnosis of goat encephalitis arthritis, using the nested PCR method, a fragment of the expected size was amplified for 37 of the 100 samples processed.

The correlation of the two methods, serologically by AGID and molecular testing by the nested PCR method, shows of the 100 samples tested from the goats studied, for 30 samples with a positive result in the AGID test of 86.67% respectively a number of 26 samples obtained positive results in nested PCR and 13,33%, respectively a number of 4 samples obtained negative results in nested PCR. In the 70 seronegative samples at 84,29% and 59 samples respectively negative results were obtained at nested PCR and at 15,71% respectively in 11 samples positive PCR results were obtained. When the AGID test is used as the only method for CAEV diagnosis, a

high rate of false-negative results (15,71%) is observed. Thus, taking into account the methods used to determine the status of infection with the *caprine arthrithis encephalitis virus* in the 100 goats, there was a higher specificity of the AGID tests of 81,08%, respectively 30 positive results from the total of 37 obtained and a higher sensitivity to nested PCR, a number of 11 seronegative animals were false negative.

The last subchapter comprises the phylogenetic analysis of the nucleotide sequences obtained from viral amplification and sequencing revealing a similarity percentage of 96,3-94,8%, showing the belonging to the prototype SLVs, the phylogenetic group B, the subtype B1, the specific *caprine arthritis encephalitis virus*.

Chapter XII **"Strategy for the surveillance and control of the *caprine arthrithis encephalitis arthritis virus* infection"** sets out the strategic surveillance and control measures adopted to reduce or eliminate the losses caused by the disease in order to limit as far as possible the incidence of the disease in goats.

In Chapter XII - **"Final Conclusions",** 20 final conclusions and recommendations are synthesized, with the main issues emerging from the research carried out.