PhD THESIS SUMMARY "ANATOMO-CLINICAL AND LABORATORY STUDIES IN OUTBREAKS OF BLUETONGUE IN ROMANIA"

PhD STUDENT: MIHAIL CLAUDIU DIACONU

SCIENTIFIC COORDINATOR: GABRIEL PREDOI, DVM, PhD, PROFESSOR

Key words: Bluetongue, serotype 4, Romania

In the field of the human and veterinary medicine, the worldwide application of the prophylaxis and combat methods allowed the achievement of some important successes in the fight against the contagious infectious diseases – the eradication of the human variola in 1979, the last case of the rinderpest in 2008 and the declaration of the disease's eradication in 2011, as well as the eradication campaigns for poliomyelitis and measles by mass immunization programs. Instead, the arboviruses' evolution manifested contrary trends, many arboviruses recording the emergence from the "traditional" sylvatic reservoirs and the global spread due to some anthropic type evolutionary factors – animals breeding, trade, the modification of the human habitats.

A ruminants' disease called "the bluetongue disease" and transmitted by insects of the *Cullicoides* genus was mentioned until 1940 only in South Africa but it pronouncedly spread afterwards, so after only 66 years, it reached the north latitude of 53° under climatic conditions that did not allow the survival from one season to another of the vectorial insects.

Bluetongue – is produced by viruses that fit within the *Orbivirus* genus, *Reoviridae* family that affects the domestic and wild ruminants, whose clinical and lesional manifestations of the disease are determined by the vascular lesions produced by the virus – the tissues' infarction, bleedings, edema and hypovolemic shock. The PhD thesis called "Anatomoclinic and laboratory studies in the Romanian bluetongue focuses" is structured according to the requirements in two parts, bibliographic study and personal research.

The 1st part "bibliographic study" comprises 4 chapters that describe the main bibliographic aspects about bluetongue, concerning etiology, epidemiological features, symptoms and the lesional picture. Data about the differential diagnosis which must be performed are indicated, on diseases and conditions with similar or adjacent clinical manifestations and the 3rd chapter describes the diagnosis techniques focusing on the modern laboratory techniques, used within the official control. The last chapter of this part briefly presents some information regarding the bluetongue prophylaxis and combat.

The 1st chapter "The general background", presents the data known up to this date about bluetongue. A few historical data are reminded, as well as the worldwide distribution of bluetongue along with the implication within the human and veterinary pathology of the *Orbivirus* genus viruses. The bluetongue viruses' structure and composition are presented along with the role of the viral components within the cellular replication cycle of the virus. Considering the non-contagious feature of the disease, the importance of the vectorial insects is described by briefly presenting data on the culicoides' biology and the way their behavioral aspects along with the geoclimatic factors influence the disease's epidemiology.

The pathogenetic aspects of the bluetongue viruses' replication within the susceptible ruminants' body are indicated, showing the manifestation manner of the symptomatic picture and implicitly, the general and particular aspects of the lesional picture according to the receptive species.

The immune response subsequent to the infection with bluetongue viruses is influenced by the antigenic determinants of the capsid, proteins also used in the conception of the tests for the disease's serological diagnosis. The clinical manifestation of the disease is highly variable on the receptive animals, being influenced by factors linked to the virus strain, but especially by the host's factors – species and race. The bluetongue viruses cross the species barriers relatively easy, a proof in this regard is the carnivores' disease signals.

Upon the presentation of the symptoms and lesions, bibliographic data were used by referring to the species of susceptible domestic ruminants (bovine, ovine, caprine) and wild ruminants' species. These data from the specialized literature, refer to the predominantly asymptomatic evolutions of the disease on the bovine and caprine species along with a clinically manifested evolution, with mortality on the ovine species. Much less bibliographic data exists about the disease on the wild ruminants, but except for the isolated cases, the evolution is generally considered clinically unapparent.

The aspects of the clinical differential diagnosis, especially in case of major exotic diseases, are important because the reaction of all the involved factors in front of a possible threat of an emergent disease, must be prompt and efficient.

The **2nd chapter "Differential diagnosis in bluetongue"** presents a series of aspects of clinical and epidemiological nature of differentiation between bluetongue and other major viral diseases or other conditions of primary nature.

The laboratory diagnosis remains the major endeavor for the identification of the virus circulation in populations of vectorial insects and cases of bluetongue infections in animals.

The bluetongue diagnosis has two main purposes: the identification of the causal agent by the detection of antigen or viral genome or the isolation from the virus, as well as the antibodies detection, as a result of the conflict between the disease's causal agent and the reactivity of the host organism.

The 3rd chapter "Diagnosis techniques in bluetongue" presents the techniques and methods available within the laboratory, all provided by the Standards manual of OMSA for diagnosis tests and vaccines, methods which were applied according to a quality management system implemented according to SR EN ISO 17025/2005.

The 4th chapter "Disease prophylaxis and combat" treats these specific endeavors for a vector-borne disease, measures provided by the community legislation. The entomological monitoring may provide valuable data for the determination of the insects' genus and species and the identification of the vectors competent in the dissemination of the bluetongue viruses, and the specific prophylaxis may protect the effectives of animals from infection.

The 2nd part "Personal research" comprises 3 major chapters, each with multiple subchapters, respectively the thesis' purpose, one chapter dedicated to the materials and methods used during the research, than the interpreted results of the investigations are presented, as well as the final part of the conclusions and a few recommendations.

The purpose of the thesis is to identify the general and specific features of the bluetongue manifestation, emergent diseases in its first evolution on the Romanian territory.

The clinical manifestations of the disease were pursued on different species of receptive ruminants, a vast majority was kept in non-professional exploitations and in an extensive system.

The lesions produced by the bluetongue virus, serotype 4, were evaluated, by performing correlations with the disease susceptibility of each species of host animals and monitoring the way the cellular tropism of the virus influences the global morphopathological picture.

The diagnosis of the disease was considered, both with classical virological methods and also with the modern instruments of the viral genome detection, given the fact that the current requirements of the official control impose the accreditation according to the international standards in force.

The behavior of the serotype 4 bluetongue virus was studied during the replication on permissive cellular lines, by monitoring the specific aspects of the resulted cytopathic effect and the ultrastructural aspects of the virions were studied by electronic microscopy, evaluating the way this technique may be used in the primary diagnosis of the emergent diseases.

The 5th chapter "Material and methods" presents the practiced types of test samples/matrices as well as the anatomoclinic and laboratory investigation methods for the bluetongue cases. The investigations were performed at the National Reference Laboratory for Arboviruses within The Institute for Diagnosis and Animal Health of Bucharest, as well as within the county sanitary veterinary and food safety laboratories according to the capability for the diagnosis methods and the regulations in force regarding the assignments within the official control.

Investigations and evaluations were performed on a number of 242 cadavers of different species of susceptible ruminants, 2125 organ sets, 3033 blood samples collected on anticoagulant treatment and 54056 samples of blood serum.

The animals infected with serotype 4 bluetongue virus were subject to clinical exams, by tracking the symptoms manifested by each species of receptive animal, and the necropsical examinations and the organs' evaluation allowed the prominence of the lesions and the observation of these morphopathological lesions' correlations with the symptomatic picture.

The diagnosis and supervision methods for the bluetongue determined by the serotype 4 virus, were applied within the laboratory. First, the methods provided within the OMSA Manual were used, for the disease diagnosis, respectively methods for the viral genome detection both for the serogroup and for serotype 4, as well as methods for antibodies detection – ELISA immunoenzymatic tests and virus neutralization tests. Also, tests of classical virology were used – virus isolation and also electronic microscopic investigations.

A series of coloration methods specific to histopathology were applied (HE, HEA, Martius Scarlet Blue, Gomori, Pappenheim) on sections of tissues/organs sampled from the cases of disease, subject to the microtomy and paraffin inclusion technique.

The 6th chapter "Results and discussions" presents in a detailed manner the results of the investigations performed on the studied samples.

The clinical tests and evaluations allowed the prominence of different categories of clinical symptoms and their share of all the considered cases.

The global interpretation of these data confirmed a typical evolution of the disease produced by the serotype 4 bluetongue virus on small ruminants (clinically manifested on ovine and poorly expressed on caprine) but atypical for bovine, with severe clinical signs.

If in the specialized literature, for the wild ruminants, the disease's evolution is predominantly asymptomatic, the evolution on the Cervidee family seems to confirm these data from the literature, less the situation described for aurochs, for which a severe evolution was detected, similar to the bovine evolution.

In case of anatomopathological tests, the correlation of the lesions with the gravity of the clinical symptoms was observed, the lesional picture is generally marked by the result of the vascular modifications induced by the virus on the entire organism's level, on the one hand due to the pathological phenomena of affecting the endotheliums of the small vessels within the tissues, with the subsequent appearance of the vascular thrombosis and the irrigated tissues' infarcts and on the other hand, by the increase of the vascular permeability that generates the tissues' edema production.

The antibodies detection performed by immunoenzymatic screening tests allowed the identification of the animals that went through the disease while the virus neutralization tests confirmed the serotype origin of the respective antibodies.

The isolation of the causal agent on permissive cultures for the viral replication, highlighted the cytopathic effect specific to bluetongue viruses and allowed the confirmation of an aspect which was described relatively recent by the literature, respectively the bluetongue viruses capacity of blocking the cellular cycle in mitosis and the induction of some aberrant mitoses.

By classical histopathological colorations, the intracytoplasmic points of the viral replications were highlighted – including viral, and the transmission electronic microscopy provided ultrastructure data that allowed the genus identification of the disease's causal agent.

Thus, the detection method for the viral genome by Real Time RT-PCR represents the basic test in the identification of the bluetongue virus infection, being applied both on blood samples with EDTA from the living animals, as well as on samples from the organs collected from the dead animals.

The correlation of these tests with the serological ones allowed the obvious determination of the infection stage for the living animals. Thus, the real image of the result of the viruses replication in tissue and organs was highlighted by the classical histopathological techniques, the

pathogenetic effects' sequence is obvious, starting from the lesion of the small vessels' endotheliums to the hemorrhagic aspect that characterizes the typical bluetongue evolution on the studied receptive species.

The 7th chapter "Conclusion and recommendations" comprises 38 conclusions drawn during and following the investigations performed on the effectives of animals in Romania, between 2014 and 2015 of the bluetongue determined by the serotype 4 virus. A number of 4 recommendations are also indicated, referring not only to the supervision and diagnosis endeavors for bluetongue but also considering wider objectives, as well as the increase of the laboratory capability for the identification of the emergent diseases, the application of supervision means adapted to the regional epidemiological context and also the increase of the awareness and involvement level of the veterinary medical personnel in the disease's surveillance activities.