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FACULTY OF VETERINARY MEDICINE
DOMAIN VETERINARY MEDICINE
SPECIALIZATION INFECTIOUS DISEASE

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ABSTRACT

RESEARCHES REGARDING BLUETONGUE

Thesis to obtain the scientifical title of
„Doctor in Veterinary Medicine"

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IAŞI
-2010-

ABSTRACT

Bluetongue, also known as the sheep "painful nose" is a noncontagious acute infectious disease transmitted by insects (arboviroses), sheep specific, but affects all ruminants, including goats, cattle, cervidee and the vast majority of species of African antelope and numerous species of artiodactiles, namely camelidae (camel, alpaca, guano and Vicuña). Does not affect horses or pigs. Although most affected sheep, cattle are the main reservoir of the virus and are important in disease epidemiology.

The disease is characterized by fever, catharral and ulceronecrotic inflammation of the nose, mouth mucous surfaces and coronary band, ulcerative stomatitis, sometimes gives tongue purple-blue color, and inflammation of podophilos tissue.

Thesis entitled „***Researches regarding Bluetongue***" is spread over 180 pages and is made in accordance with current legal provisions, the two main parts: the first part entitled „***Actual state of knowledge***„ includes 48 pages, 7 tables and 16 figures and the second „***Personal contributions***" occupies 108 pages, 36 tables, 41 figures, for better presentation of content.The first part consists of four chapters which are summarized, information from literature on the subject of the sentence which was subsequently used to interpret and compare data obtained in the second part. Also are five annexes with 33 photos.

The first chapter entitled "***Bibliographical datas regarding Bluetongue history, distribution and importance***" presents data on the first descriptions of disease and etiological agent, in the distribution and importance of this disease.

Bluetongue has been described in Africa, the first reports dating back to 1876, once with increasing Merino sheep breed in South Africa, but was recognized in most countries of the tropics and southern tropics. Epidemiological, clinical and immunoprophylactic studies were made by Spreull (1905), Theiller (1905-1906), when the viral nature of the disease and an effective method of vaccination were established. The disease causes economic damage by mortality, prolonged convalescence, decreased milk, wool, meat production, breeding disorders (abortion, viable products or congenital anomalies).

The second chapter entitled ***Aspects of etiology, epidemiology and pathogenesis in Bluetongue*** is structured in three chapters, summarizing key data from the literature regarding the viral morphology, general characteristics of orbiviruses, structure and function of proteins VP2 and VP5, cultivation and viral replication, virus resistance to the action of various factors. In the second chapter are data on responsiveness, the main source of infection and transmission and epizootic dynamic. The last chapter describes the pathogenesis, as detailed in importance of culicoid vectors in disease transmission from infected animals to other susceptible.

In the third chapter entitled ***Clinical signs and morphopathology in animal Bluetongue virus infections*** are treated issues related to symptoms and lesions produced in susceptible species.

In sheep, the disease progresses acute, subacute and aborted form. Symptomatology is characterized by intense hyperthermia 42°C, depressed mood, sialorea, hyperemia of mouth, nose, conjunctiva and skin of the face, ears, feet. The tongue is highly swollen, violet color, hence the name of Bluetongue disease. Animal death occurs in 8-10 days or 3-5 weeks after bacterial complications or starvation.

In cattle, BTV 8 infection progresses inaparent or benign. Fever, mucopurulent oculonazal catarrh, swelling and cyanosis of the tongue, death in 24-48 hours, the intense dehydration, due to foot problems. Inaparente clinical infections can occur with him serotype bovine and other species.

Another chapter presents pathological changes that may be present at the digestive, respiratory muscles, circulatory system, and sometimes urogenital apparatus.

In chapter 4, titled ***Diagnosis, monitoring and control of Bluetongue virus infection*** are presented the main diagnostic methods, referring to the etiologic and differential diagnosis and measures to limit spread of infection or entry into the free areas.

In ***chapter 5*** are presented the aims and objectives of thesis. The entire study periode, the research took into account five main goals:

- Presentation of results of passive and active surveillance during the years 2004-2010 (2003 Pilot);
- Presentation of results from the study of a event related to the death of an imported cattle from a restricted area for Bluetongue;
- Presentation of results from surveillance and culicoizi vector control in Neamț County;
- Presentation of results from serological monitoring of sheep in trashumance;

- Presentation of results from studies conducted under the ROPATOSILV, institutional cooperation program for diagnosis, prevention and control of medical conditions with major epidemiological significance in wild animals.

Chapter 6 presents the results of research on the significance of laboratory tests in diagnosis of Bluetongue. The first chapter presents the results of serological tests carried out under supervision, given that Romania is a free country for this disease.

Serum samples obtained from susceptible species (cattle, sheep, goats, wild ruminants) were tested for antibodies against Bluetongue virus. For serological tests were used: competitive ELISA kit for detection of antibodies to specific VP7 protein of Bluetongue virus (Institute Pourquier), ELISA kit for detecting Bluetongue antibodies (VMRD INC., USA), ELISA kit for detecting Bluetongue antibodies (BTV Ingezim).

Serological tests were performed in 2007, in Neamț County, a herd of cattle on 99910 cows, heifers, one owner with 21580 horses, and a flock of 208526 sheep. In serological investigations carried out in 2007, in the 1231 serum samples of cattle and 411 sheep serum samples was not identified any seropositive animals.

In 2008, serological surveillance was conducted on a total of 1194 samples, including samples taken from 900 cattle, 292 sheep taken from two samples taken from goats. The area of coverage was 38 target locations for cattle and 36 for sheep. Also, in 2008 were tested serum samples from 203 cattle imported and all results were negative.

In the period January to December 2009 were tested a total of 1505 samples, of which 1186 samples taken from cattle, 280 from sheep, 35 from goats and four samples taken from other susceptible species. The area surveillance was the 18 target villages for cattle, 17 sheep target locations, one location for goats and a reserve.

In serological investigations carried out in 2009, organized on target species (cattle, sheep, goats, bison), none of the evidence was not tested positive, confirming allowance herds from which they came, in terms of the presence of Bluetongue virus. During January to April 2010 were tested a total of 311 samples, of which 299 samples taken from cattle, nine samples collected from sheep and three samples taken from goats.

Surveillance area was the 10 target villages for cattle, five for sheep and only one location for goats. Serological investigations carried out so far, organized by target species (cattle, sheep, goats) have revealed that none of the evidence was not tested positive, confirming that it is this careful monitoring animals susceptible to Bluetongue virus infection contribute to the maintenance-free country status.

Virological monitoring was performed both on blood samples collected on anticoagulant (EDTA), from viremic animals imported from restricted zones or in areas where were vaccinatied and samples of organs from an animal imported into Romania from a European Union country, declared restricted zone for Bluetongue. Examinations were performed by RT - PCR in IDSA Bucharest.

In 2009, a special event was represented by a group of 20 cattle imported from an EU country, a restricted zone for Bluetongue. An animal presented altered general condition expressed by fever, respiratory events and mild nasal congestion, crusting around the mouth and nostrils. Evolution was rapid and the animal was found dead. Virological examinations were performed by RT - PCR on samples taken from this animal.

In parallel samples were taken for bacteriological and virological examinations and tests of central nervous system (CNS) to exclude the diagnosis of bovine spongiform encephalitis (BSE). Following investigations were obtained the following results: negative bacteriological, virological the set of organs - spleen, lung, blood and submandibular lymph - RT-PCR negative for Bluetongue, examination for BSE by ELISA - negative.

In ***chapter 7*** are presented the results of investigations on the monitoring of vectors with the role of Bluetongue virus transmission. Entomological monitoring was done by installing a fixed traps to catch insects in monitoring programs, followed by culicoid identification, preservation and preparation of their entomological records for each catch.

Identification of species of the genus *Culicoides*, in 2004, was made during August-October on 15 catches insects preserved rough, caught. Together with Dr Aurelia Ionescu and Dr. Rudy Meiswingel I attended the installation of mobile traps in different locations. Samples were sent for identification to IDSA Bucharest.

Monitoring delivery is used to obtain data on the distribution of vectors in a given territory to determine the seasonal abundance of insect vector species in a given area, the purpose of isolating and identifying potential they circulated virus and transmitted.

Identify species of the genus *Culicoides* was carried out since 2004, when in Neamț County were detected Bluetongue virus vectors, by moving the location of light traps to deposit of stallions and ECT farm. Studies made by Dr. Rudy Meiswingel during April-May 2004, showed that *C. obsoletus* predominate in Neamț County, compared with *C. pulicaris*, and if one takes into account the competence of the vectors

Thus, in 2005, was purchased and installed a fixed trap at DT deposit of stallions standing animal shelter no. 4, for vector monitoring, trapping and currently operating with the same purpose of taking delivery. Since 2007 has been implemented monthly serological

monitoring cattle and sheep under EU law, EU co-financed 50% of the serological and virological. The final conclusion is that vectors monitoringtook place in 2005, 2006, 2007, 2008, 2009 and continuing into 2010 and serological and virological monitoring was conducted in 2007, 2008, 2009 and continuing into 2010. These two types of monitoring overlapped and joined, from 2007, then continued in the years 2008, 2009 and 2010.

From 2005 to 2006 were examined a total of 96 catches, fixed trap location being stallion deposit. Surveillance of vectors point entomological and virological view was held under the strategic plan.

During 2009there were captured a number of 5856 vectors, but only 2556 (43,65%) have been identified as *Culicoides*. Following morphological examination, 2475 (96,83%) species were identified as *C. obsoletus* and 81 (3,17%) as *C. pulicaris*. It was also noted that 1594 (62,36%) of insects are females parous.

The data obtained from monitoring the 2010 vectors to date have revealed that from 121 captured insects, only 4 (3,3%) were identified as *Culicoides*, all the species being *C. obsoletus*. Also, only a parous female was identified.

In Chapter 8 are presented strategic measures of supervision and control of Bluetongue in Romania. ECT Farm has three sheep farms, with different locations. In sheep transhumance were tested serologically by ELISA in the Bluetongue surveillance program.

Blood samples were collected in two separate periods, in October 2009 and May 2010. Serological and virological monitoring were conducted on samples from three sheep farms in the surrounding villages ECT.

Were tested 15 and 16 serum samples collected from Karakul sheep breed, White Turcan and rams to highlight the Bluetongue virus antibodies, provided with negative results. Never justified virological testing by RT-PCR.

Regarding wild ruminants a collaboration with Romsilva was established.

With the start of hunting season in wild ruminants, at harvest of trophy, selection and other actions that are laid down in Hunting and protection of cinegetic fund Law, no. 407/2006, published in Official Gazette 407/2006, updated, laboratory tests (virology and molecular biology) were performed in wild ruminants shot to collect useful data for epidemiological and risk analysis.

This objective has been possible through close cooperation with forestry government and hunting associations. The Ropatosilv program - institutional cooperation program for diagnosis, prevention and control of medical conditions with major epidemiological

significance of wild animals were colected blood samples and organs from two rafters in 2009.

Samples were transported to Bucharest IDSA respecting the conditions of sample transport and, from economic reasons, virulogical exam was made by inoculating embryonated eggs. The s analized samples were negatives, so we can conclude that they were free of Bluetongue virus.

În chapter 19 final conclusions and 4 recommendations are briefly presented.

The thesis also includes an annex giving the figures representing various stages of work.