

SUMMARY

Key words: *epidemiology, virus, bluetongue, domestic ruminants,*

The PhD thesis, entitled "**Research on epidemic surveillance of blue tongue disease (Bluetongue) in some counties from South-East Romania**", is structured in two main parts. **Part I**, "*The current state of knowledge*", extended on 55 pages, represent a sunthesis of the information extracted from the literature on the paper topic. **Part II**, "**Personal contributions**", which comprises a total of 137 pages, presents the results of the research undertaken during the doctoral studies.

The first part, "*The current state of knowledge*" is a sintetic review of the current issues regarding *Bluetongue* disease and an updated epidemiological situation of the disease, in Europe and Romania.

The first chapter entitled "**Bibliography data on Bluetongue disease**" presents the most important aspects regarding the description and etiology of the disease.

In the **second chapter**, entitled "**The epidemiological situation of Bluetongue disease in Europe and Romania**" the main effects produced by *Bluetongue* in Europe and Romania are presented. *Bluetongue* has a significant economic impact, mainly due to the effect of the disease on animals (morbidity, mortality, insufficient reproduction, decreasing of milk production and weight gain) and, in particular, disruption of international trade in animals and animal products.

Part II, "**Personal contributions**", is structured in six chapters (III - VIII), each one of them comprising the detailed studies performed on the proposed research topic, materials, methods and the results obtained. In the last chapter the general conclusions detached from the overall analysis are systematized.

Chapter III present the "**Purpose of the research, objectives and organizational framework**". In the last decades, Europe has become an increasingly exposed space for *Bluetongue*. The causes underlying the spread of the disease are diverse and, as a result, the distribution was almost uniform across the continent, reaching even in Northern Europe. In Romania, the first case of *Bluetongue* was reported on August 22, 2014 in Buzău County. After the first notification, the number of reported cases showed a fast evolution. Thus, just 5 days after the confirmation of the first case, ANSVSA confirmed the evolution of 28 outbreaks in Buzău, 73 cattle and 5 sheep with clinical forms of *Bluetongue* being involved. In just 7 days after the alert, the disease spread to neighboring counties, with 49 outbreaks reported. On September 4, *Bluetongue* spread to 12 counties, triggering 63 outbreaks of disease and 447 affected animals. The fast distribution of the *Bluetongue* virus in August-September was the consequence of the favorable climatic conditions for the proliferation of the culicoid vectors existing at that time in the region. In these circumstances, the possibility that the *Bluetongue* virus could persist and spread in all regions of the country was a certainty. In order to achieve the epidemiological situation that started in 2014, we set out to participate at the blood samples harvesting, the evaluation and laboratory testing of the samples, the centralization and analysis of the data obtained by surveillance of *Bluetongue* in Vrancea, Galați, Brăila and Tulcea, during 2014-2017, three of these counties being adjacent to the area where the first outbreak of disease evolved.

In order to achieve this goal, during the mentioned periode, the four counties from the South-East region of Romania were monitored and the following objectives were established: serological surveillance of the *Bluetongue* disease in domestic ruminants; virological surveillance of *Bluetongue* disease in domestic ruminants; capture and identification of the *Bluetongue* virus transmitting vectors

and evaluation of the clinical and lesional aspects that have evolved in ruminants with Bluetongue virus infections.

Chapter IV, entitled "*Investigations regarding the serological surveillance in the Bluetongue disease in ruminants in the South-East region of Romania*" aimed to carry out epidemiological investigations regarding the incidence of *Bluetongue* virus (BTV) antibodies in the serum samples obtained from the ruminants from the 4 monitored counties. During 2014-2017, in the South-East region of Romania 12846 receptive animals tested by serological methods revealed a variable incidence of BTV antibodies according to the period and the geographical area. In 2014, Vrancea County had the highest incidence (58.5%) of seropositive animals, followed by Galați (3.21%), Brăila (2.24%) and Tulcea (0.58%).

In 2015, the incidence of positive *Bluetongue* animals was in a slight regression, decreasing to 32% in Vrancea, 2.74% in Galați, and 0% in Tulcea, except for Brăila county, where the share increased to 6.62%.

In 2016, Galați County was noted with a significant increase in the incidence from 2.74% (2015) to 15.35% (2016), compared to Vrancea County, where the incidence has decreased significantly from 32% (2015) at 10.48%, and in Brăila County, the incidence index (6.21%) was close in value to the previous year (6.62%).

In 2017, the incidence of seropositive animals suffered a significant decrease from 15.35% (2016) to 0.81% (2017) in Galați County, from 6.21% (2016) to 3.35% (2017) in Brăila County, and the incidence increased from 10.48% to 15.85% in Vrancea County. In Tulcea County, this year, no seropositive animals were identified for *Bluetongue* virus. The variability of the incidence index, within the serological monitoring, showed an oscillating character and the ability of the virus for propagation in the South-East region of Romania. Serological monitoring performed in domestic ruminants between 2014-2017 revealed the continuous presence of specific antibodies for the *Bluetongue* virus in this region.

Chapter V, entitled "*Virological investigations in Bluetongue*" contains the data obtained following the virological examination by the *Real-Time Polymerase Chain Reaction Assays* (RT-PCR) in order to identify the viral genome in the domestic ruminants from the 4 target counties and to establish the index of incidence in the South-East region of Romania. During 2014-2017, 1024 seropositive animals were diagnosed with *Bluetongue* and the blood samples were submitted to virological examination.

In **Vrancea County**, between 2014 and 2017, the virological examination was performed on blood samples harvested from 429 seroconversion animals from the sentinel farms and 370 animals suspected of *Bluetongue*. In 2014, the tests confirmed the presence of the viral genome in 47.83% (n = 77) bovine blood samples (n = 161) and 50% (n = 102) of sheep blood samples (n = 204), taken from animals seropositive with suspected disease. Between 2015 and 2017, the *Bluetongue* viral genome was not identified in seropositive animals from Vrancea County. Confirmation of the presence of *Bluetongue* virus in cattle and sheep, correlated with the results of entomological surveillance in the area of this county, led to the declaration of 179 *Bluetongue* outbreaks reported in 43 localities from Vrancea county.

In **Galați County**, the virological examination was performed on blood samples collected from 61 seroconversion sentinel animals and 35 animals suspected of *Bluetongue*, with positive BTV antibodies. Virological diagnosis confirmed the presence of the viral genome in 50% (n = 2) of bovine blood samples (n = 4) taken from seropositive animals with suspected disease, identified in 2014. During 2015-2017, *Bluetongue* viral genome was not identified in seropositive animals from Galați County. Confirmation of the presence of *Bluetongue* virus in cattle and sheep, correlated with the results of entomological surveillance in the area, led to the declaration of 2 *Bluetongue* outbreaks, in Galați County, in 2014.

In **Brăila county**, the virological examination by Real-Time-RT-PCR technique was performed on blood samples collected from 114 seroconversion sentinel animals and 11 animals suspected of *Bluetongue*, with positive BTV antibodies. Virological diagnosis confirmed the presence of the viral genome in 66.66% (n = 2) of bovine blood samples (n = 161) from seroconversion cattle

and 100% (n = 3) of blood samples from seropositive cattle with suspected disease, identified in 2014. Between 2015 and 2017, the *Bluetongue* viral genome was not identified in seropositive animals from Brăila county. Confirmation of the presence of *Bluetongue* virus in cattle and sheep, correlated with the results of entomological surveillance in the area, led to the declaration of 4 *Bluetongue* outbreaks in 2014.

In **Tulcea county**, in 2014, the virological examination was performed on 4 blood samples harvested from 2 bovine and 2 sheep with seroconversion. Virological diagnosis confirmed the presence of the viral genome in 50% (n = 1) of cattle (n = 2) and 50% (n = 1) of sheep (n = 2) with seroconversion. During 2015-2017, *Bluetongue* viral genome was not identified in seropositive animals. Confirmation of the presence of *Bluetongue* virus in cattle and sheep, correlated with the results of entomological surveillance in the area, led to the declaration of 2 *Bluetongue* outbreaks, in 2014.

The infection with the *Bluetongue* virus is not persistent. Due to their pathogenicity, the infection with *Bluetongue* virus may not be diagnosed for a certain period of time, but meanwhile the disease is still present and evolves and the infected animals are sources of infection for the culicoid insects.

In *Bluetongue* epidemiology, cattle play a particularly important role, due to prolonged viremia in the absence of clinical signs of the disease. However, there is no explanation for the very high morbidity rate in cattle in Romania.

Chapter VI, entitled "*Investigations on the clinical and anatomopathological aspects of the Bluetongue disease in the domestic ruminants in the South-East region of Romania*", presents the clinical and lesional aspects of the infection with the *Bluetongue* virus, in the cattle, sheep and goats from the 4 monitored counties. In cattle, the disease evolves frequently without obvious clinical features, with the exception of serotype 8 (BTV8) infection which is clinically expressed with fever, cataract or ulcero necrotic inflammation in the nasal, oral and coronary mucosa. However, in Romania the BTV4 serotype was identified, clinically and lesionally expressed as the BTV8 serotype. During the research periode, 1437 domestic ruminants were clinically examined, 418 (29.08%) showing symptoms similar to those specific to the *Bluetongue* virus infection.

The clinical picture in *Bluetongue* virus infection **in cattle** was representative: 100% (n = 209) with conjunctival mucosal hyperemia, 98.56% (n = 206) with epiphora, 98.56% (n = 206) with sialorrhoea, 97.6% (n = 204) gingival ulcerations, 95.7% (n = 200) with swelling and cyanosis of the tongue, 95.7% (n = 200) with nasal edema, 90.43% (n = 189) with ulcers of the nasal mucosa, 88.90% (n = 186) with muco-purulent discharge, 80.38% (n = 168) circumscribed necrosis, ulcers in the uterus, 76.07% with marked weakening, 73.2% (n = 153) congestion of the coronary area, 57.9% (n = 121) with nervous phenomena (torticollis), 48.32% (n = 101) with diarrhea, 45.93% (n = 96) with immobility, 44.01% (n = 92) with exongulation, 13.88% (n = 29) with ulcers on the posterior areas and 5.26% (n = 11) with abortions.

The clinical picture in **sheep** was similar to that in cattle, evolving to 94.66% (n = 195) with prostration, 100% (n = 206) with muco-purulent nasal secretions, 100% (n = 206) with edema and nasal ulceration, 97.10% (n = 200) with cyanosis of the oral mucosa, 100% (n = 206) with ulceration of the lips, 100% (n = 206) with ulcers and necrosis circumscribed in the oral mucosa, 99.5% (n = 205) with sialoree, 53.4% (n = 110) with epiphora, 99.5% (n = 205) swelling and cyanosis of the tongue, 96.6% (n = 199) were incapacitated and 64.10% (n = 132) showed marked weakness.

Infection with the *Bluetongue* virus **in goats** had a reduced clinical expression, the following being observed: 100% (n = 3) with prostration, 100% (n = 3) with muco-purulent discharge, 100% (n = 3) with edema and nasal ulceration, 66.6% (n = 3) with oral mucosal cyanosis and 100% (n = 3) with lameness.

The lesion picture was described only in bovines slaughtered by necessity, where the incidence of the aspects varied according to the clinical evolution: in 100% (n = 8) bovine animals, enlarged petechiae were identified in the subcutaneous connective tissue, 75% (n = 6) presented congestion and bleeding at the anterior pillar of the rumen, 100% (n = 8) showed splenic hypertrophy, 87.5% (n = 7) with splenic multifocal hemorrhages and arterial pulmonary hemorrhage. In 87.5% (n = 7) congestion,

edema and cyanosis of the larynx and pharynx were observed and 75% (n = 6) of animals showed pulmonary edema and tracheo-bronchial foam.

Correlating the epidemiological results obtained by the serological, virological and entomological investigations, we can conclude that the *Bluetongue* virus is in a latent state, which did not allow its identification during 2015-2017. In the same time the BTV can be reactivated under the influence of predisposing factors: the presence of vector insects from genus *Culicoides* and increasing number of the receptive animals.

Chapter VII, entitled "*Investigations regarding the entomological surveillance of the Bluetongue virus transmitting vectors*" presents data obtained from the entomological monitoring, that followed some objectives: evaluation of presence and incidence of *Culicoides* insects, identification of the culicoid species and the evaluation of the vector activity in the studied area. Because the monitored counties have various relief forms, both in terms of altitude and shape, with numerous natural reserves, flora and fauna create complex habitats for the survival and propagation of these vector insects.

In **Vrancea County**, hematophagous insects from *C. obsoletus* complex and *C. pulicaris* complex were identified, considered vectors for the transmission of the *Bluetongue* disease. **In 2014**, the incidence of *Culicoides* insects was 1.76% (n = 8), from which 37.50% (n = 3) were *C. obsoletus* and 62.50% (n = 8) were *C. pulicaris*. Vector activity was reported in September-October at thermal values between 6°C and 12°C for *C. pulicaris* and 7°C-12°C for *C. obsoletus*. **In 2015**, the incidence of *Culicoides* insects was 13.54% (n = 765); 79.95% (n = 604) were *C. obsoletus*, 12.02% (n = 161) were *C. pulicaris*. and 9.02% (n = 69) were non-vector culicoids. Vector activity of the culicoids was reported between May and September, at thermal values between 15°C and 33°C for *C. obsoletus* and 12°C and 23°C for *C. pulicaris*. **In 2016**, the incidence of culicoid insects was 15.38% (n = 1182). Of these, 61.50% (n = 727) were *C. obsoletus* and 38.50% (n = 455) were *C. pulicaris*. Vector activity of the culicoids was reported between April and September, at thermal values between 7°C and 36°C for *C. obsoletus* and 7°C and 32°C for *C. pulicaris*. **In 2017**, the incidence of *Culicoides* insects was 0.56% (n = 8) and of these 50% (n = 4) were *C. obsoletus* and 50% (n = 4) were *C. pulicaris*. Vector activity of the *C. obsoletus* and *C. pulicaris* culicoids was reported during July - September, at thermal values between 19°C and 34°C.

In **Brăila county**, hematophagous insects from *C. obsoletus* complex, *C. pulicaris* complex, and *C. nubeculosus* were identified and considered vectors for the transmission of the *Bluetongue* disease, with different incidence depending on the years. In 2014, the incidence of *Culicoides* insects was 4.16% (n = 175), from which 45.71% (n = 80) were *C. obsoletus*, 20.57% (n = 36) were *C. pulicaris*, 8.57% (n = 15) were *C. nubeculosus* and 22.14% (n = 44) were "non-vector" *Culicoides* species. Vector activity of the culicoids was reported in August-November at thermal values between 3°C and 31°C, for *C. obsoletus*, in April-September at thermal values between 11°C-30°C; for *C. pulicaris*, in September-November at thermal values between 3°C and 30°C. In 2015, the incidence of *Culicoides* insects was 1.61% (n = 641) and 26.83% (n = 172) were *C. obsoletus*, 72.38% (n = 464) were *C. pulicaris*. Vector activity of the culicoids was reported during April-September, at thermal values between 8°C and 34°C for *C. obsoletus* and during April-November at thermal values between 5°C and 34°C for *C. pulicaris*. In 2016, the incidence of culicoid insects was 4.15% (n = 1182), and of these, 21.25% (n = 112) were *C. obsoletus*, 77.22% (n = 407) were *C. pulicaris* and 1.51% (n = 8) were "non-vector" *Culicoides* species. Vector activity of the culicoids was reported during May-September, at thermal values between 7°C and 34°C for *C. pulicaris*, during June -September when the thermal values were between 7°C and 34°C for *C. obsoletus* and in June-July at the thermal values between 13°C and 33°C "non-vector" *Culicoides*. In 2017, the incidence of *Culicoides* insects was 1.33% (n = 62) and 40.32% (n = 25) were *C. obsoletus*, 59.68% (n = 37) were *C. pulicaris* and the vector activity of the *C. obsoletus* and *C. pulicaris* culicoids was reported during the period May-October, at thermal values between 11°C and 36°C.

In **Galați County**, hematophagous insects from *C. obsoletus* complex, *C. pulicaris* complex, and *C. nubeculosus* have been identified as vectors for the transmission of the *Bluetongue* disease. In 2014, the incidence of *Culicoides* insects was 7.73% (n = 249), of which 84.33% (n = 210) were *C.*

obsoletus, 10.84% (n = 27) were *C. pulicaris*, 2.83% (n = 7) were *C. nubeculosus* and 2.0% (n = 5) were "non-vector" *Culicoides* species. Vector activity of the culicoids was reported in April, at thermal values between 11⁰C and 20⁰C for *C. pulicaris*, in September at thermal values between 17⁰C and 29⁰C for *C. obsoletus*, in April and November at thermal values between 3⁰C and 30⁰C for *C. nubeculosus*. In 2015, the incidence of *Culicoides* insects was 0.93 (n = 165) and 99.40% (n = 164) were *C. pulicaris*, 0.6% (n = 1) were *C. obsoletus*. Vector activity of the culicoids was reported only in April, at thermal values between 5⁰C and 18⁰C for *C. obsoletus* and in April-October with thermal values between 5⁰C and 33⁰C for *C. pulicaris*. In 2016, the incidence of culicoid insects was 6.04% (n = 732), and of these 60.24% (n = 441) were *C. obsoletus*, 39.30% (n = 287) were *C. pulicaris* and 0.55% (n = 4) were *C. nubeculosus*. Vector activity of the culicoids was reported during April-November at thermal values between 10⁰C and 28⁰C for *C. pulicaris*, during June-August when the thermal values were between 14⁰C and 28⁰C for *C. obsoletus* and in July at thermal values between 21⁰C and 28⁰C for *C. nubeculosus*.

In **2017**, the incidence of *Culicoides* insects was 14.81% (n = 1050) and of these 99.14% (n = 1041) were *C. obsoletus* and 0.85% (n = 36) were *C. pulicaris*. Vector activity of the *C. pulicaris* culicoids was reported in May when thermal values were between 13⁰C and 19⁰C and during August-October when the thermal values were between 6⁰C and 28⁰C. *C. obsoletus* carried out its vector activity in July-August when the thermal values were between 11⁰C and 28⁰C. The statistical analysis shows the tendency to increase the density of culicoids in Galați County area. *C. obsoletus* and *C. pulicaris* are constantly in a cohabitation relationship, being the main vector species present in Galați County.

In **Tulcea County**, hematophagous insects from *C. obsoletus* complex and *C. pulicaris* were identified as vectors for the transmission of the *Bluetongue* disease. In 2014, the incidence of *Culicoides* insects was 13.54% (n = 1197), of which 64.66% (n = 774) were *C. obsoletus*, 25.30% (n = 303) were *C. pulicaris* and 10.2% (n = 120) were "non-vector" *Culicoides* species. Vector activity of the culicoids was reported from April to October, at thermal values between 8⁰C and 34⁰C for *C. obsoletus*, between July and September-October when thermal values were between 14⁰C and 29⁰C for *C. pulicaris*. In 2015, the incidence of *Culicoides* insects was 21.25 (n = 2056) and of these 71.30% (n = 1466) were *C. obsoletus*, 13.13 % (n = 270) were *C. obsoletus* and 15.56% (n = 320) were "non-vector" *Culicoides* species. Vector activity of the culicoids was reported during April-November, at thermal values between 7⁰C and 34⁰C for *C. obsoletus* and during April-September at thermal values between 8⁰C and 34⁰C for *C. pulicaris*.

In **2016**, the incidence of *Culicoides* insects was 12.26% (n = 1865), and of these 62.14% (n = 1159) were *C. obsoletus*, 27.30% (n = 509) were *C. pulicaris* and 10.56% (n = 197) were "non-vector" *Culicoides* species. The vector activity of the species *C. obsoletus* was reported during the period May-October when the thermal values were between 8⁰C and 34⁰C. The vector activity of the species *C. pulicaris* was identified during the period May-September, when the thermal values were between 12⁰C and 34⁰C. In **2017**, the incidence of *Culicoides* insects was 9.86% (n = 1105) and of these 60.82% (n = 672) were *C. obsoletus*, 21.26% (n = 235) were *C. pulicaris* and 17.92% (n = 198) were "non-vector" *Culicoides* species. Vector activity of *C. obsoletus* culicoids was reported in May-October when thermal values were between 10⁰C and 35⁰C and 12⁰C - 35⁰C for *C. pulicaris*. The statistical analysis performed during 2014-2017, showed the tendency of decreasing the density of the culicoids on the territory of Tulcea county. The topo-geo-climatic conditions of Tulcea County are favorable for the emergence of new vector species of the genus *Culicoides*.

Analysis of the vector incidence, related to the number of insects captured during the entire research period (2014-2017), showed that Tulcea was the territory with the highest density of culicoid insects (13.85%), followed by Vrancea (12, 9%), Galați (5.48%) and Brăila (2.3%).

The global warming phenomenon has led to the increase of multiannual temperatures and to the extension of the *Culicoides* area in România. In the same time topo-geo-climatic conditions in Vrancea, Galați, Brăila and Tulcea counties offer the region a natural focal character.

In **chapter VIII**, entitled „*Final conclusions and Recommendations*” entitled the main conclusions drawn from the seroepidemiological, virological, entomological, clinical and

anatomopathological investigations are summarized. Considering the increased risk of producing new *Bluetongue* outbreaks in the South-East region of Romania and the economic effects generated by this disease, six recommendations that can be sustained in the long term have been formulated.