Bluetongue in Europe and Romania in the Last Years

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Abstract
Beginning with 2006, in Europe had been a lot of Bluetongue (BT) outbreaks evolving like hemorrhagic disease in ruminants, caused by bluetongue virus (BTV). This paper reviews an epidemiological situation of this disease in Europe and in Romania in the last years with all health and economic problems that followed the outbreaks. For the first time described in 1905 in Merino sheep from South Africa, BTV is an *Arbovirus* isolated from wild and domestic ruminants, with sublinical to fatal symptoms of disease. The name Bluetongue derived from necrosis, cell injuries with vascular thrombosis, oedema, haemorrhages that make the tongue cyanotic, with a blue colour. By historically point of view BTV is known to be prevalent in tropical and subtropical areas between 35° S and 45° N, but many outbreaks were than reported in further northwards, an emergence may be caused by climate changes, when a high temperature contributes to both distribution and competence of certain species of *Culicoides* midges, known to be the insect vector of BT disease. With the exception of Antarctica BTV was been isolated on every continent. From 1,400 species of *Culicoides* throught the world, only about 30 species have been discovered to be competent biological vectors. There are more regions in Europe believed previously to be not a risk for bluetongue evolution but the situation since 2006 demonstrates the contrary.

Key words biological vector**,** bluetongue, epidemiological situation, outbreaks.

1. Introduction

Bluetongue (BT), also described as epidermal bluetongue, is a non-contagious, infectious disease characteristic of sheep, which is acute form commonly evolving [1, 2]. Bluetongue is also known as sore muzzle, malarial catarrhal fever, epizootic catarrh, and muzzle disease. Occasionally this disease can affect bovines and some wild ruminants. It is produced by an RNA virus belonging to the *Orbivirus* genus in the *Reoviridae* family that is transmitted by arthropods. For the first time, bluetongue (BT) occurred and was reported in flocks of sheep in South Africa, where it caused great losses through mortality, especially in Merino sheep. After 1940, BT was reported in Cyprus (Gambles, 1949), in Turkey (Aral, 1949), in Israel (Komorov, 1951), in the United States. Hardy in 1952, then, between 1956 and 1957, caused major epidemics in Portugal and Spain. It has also been described in Pakistan and Australia. Currently, it evolves in most tropical and subtropical countries [3, 4]. The first studies on epidemiology, anatomoclinical and immuno-prophylaxis were carried out by Spreulle in 1905, and the isolation and cultivation of the virus in order to prepare an inactivated vaccine was carried out by Theiller in 1906. Bluetongue disease, as well as other infections produced by arboviruses, commonly occur and evolve in 35-40° latitude geographic areas described in Africa, America, Australia and the Middle East. In African, American, Australian and Asian continents, there are areas where the disease

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develops endemic, with certain free areas of infection. The distribution and prevalence of infection in the regions of these continents are influenced by climatic, altitude, presence of susceptible mammals as well as the spread and activity of vectors. In some countries, such as Australia, Brazil, Japan, Canada and the West Indies, bluetongue virus (BTV) has been serologically detected in a significant number of animals without expressing characteristic clinical signs [5].

Etiology

Bluetongue disease is caused by a virus belonging to the genus *Orbivirus* of the family *Reoviridae*, subfamily *Sedoreovirinae*, according to the International Committee on Taxonomy of Viruses. The BTV genome is the linear, double-stranded RNA molecule, divided into 10 segments. The 10 segments of the genome, by polyacrylamide gel electrophoresis, according to their molecular mass, migrate to distinct areas, a property that serves to identify isolates. Under laboratory conditions, the virus can be grown on different types of cell cultures: BHK21 (young hamster kidney cells); L929 (mouse fibroblasts); Vero; Cv6136 (cells obtained from the salivary glands of the *Culicoides variipennis* insect) or endothelial cell cultures derived from cattle. Sometimes virus isolation in cell cultures only succeeds after several successive passages. The most sensitive method of isolating the BTV is by inoculating it, intravascularly, into the embryo of the hen or the sheep that did not come in contact with the virus before the inoculation. Also, the virus can be cultured, after intracerebral inoculation, in mice and infant hamsters. BTV is resistant to putrefaction and organic solvents (ether, chloroform) but is sensitive to action of acid pH (below 6) or alkaline (above 8) as well as to trypsin action. Temperatures between minus 10° C and minus 20° C decrease the virulence of the virus. In preserved blood at laboratory temperature, it lasts for up to 3 months, and in nervous tissue, preserved at + 4°C, it retains virulence up to 7 years. Virologists currently identified 27 different serotypes, of which, all but one can be genetically traced to their original geographic origin; the exception is BTV-27 due to its recent (2014) discovery. There are at least 24 serotypes worldwide, although not all serotypes exist in any one geographic area; 13 serotypes (1, 2, 3, 5, 6, 10, 11, 13, 14, 17, 19, 22, and 24) have been reported in the USA and 8 serotypes (1, 2, 4, 6, 8, 9, 11, and 16) in Europe. Distribution of BTV throughout the world parallels the spatial and temporal distribution of vector species of *Culicoides* biting midges, which are the only significant natural transmitters of the virus, as well as the temperatures at which BTV will replicate in and be transmitted by these vectors. Of more than 1,400 *Culicoides* species worldwide, fewer than 30 have been identified as actual or potential vectors of BTV to date. Continued cycling of the virus among competent *Culicoides* vectors and susceptible ruminants is critical to viral ecology. In the USA, the principal vectors are *C. sonorensis* and *C. insignis*, which limit the distribution of BTV to southern and western regions. In northern and eastern Australia the principal vector is *C. brevitarsis*, whereas in Africa, southern Europe, and the Middle East it is *C. imicola*. In northern Europe, the major vectors are species within the *C. obsoletus-dewulfi* complex. In each geographic region, secondary vector species may attain local importance. Serotypes differ by the point of view of virulence, hence the extent of clinical signs may vary. The numerous serotypes are the result of genetic shift (reassortment) and drift (mutation) from alternating passage of BTV through ruminant and insect hosts. BTV is a non-enveloped, linear, and segmented double-stranded ribonucleic acid (dsRNA) virus. There are 10 segments that code for 10 proteins, 7 structural proteins (VP_1–VP_7) and 3 nonstructural (NS_1, NS_2, NS_3/NS_3a) proteins. Two of the structural proteins (VP_2 and VP_5) make up the icosahedral capsid of the virus. Serotype is primarily determined by VP_2, the most variable of the BTV proteins, which interacts with neutralizing antibodies. The geographic origin of the serotypes is reflected in the variable sequence of the segments that make up a specific serotype's genome, allowing further classification of serotypes into topotypes. Electron microscopically examined, the viral particles have a spherical shape and a diameter between 60-80 nm. The viral envelope is composed of capsid only, and it exhibits icosahedral symmetry and is diffuse structured on three layers. The segregation and geographic distribution of serotypes belonging to the same virus resulted in the emergence of 3

major serotypes in North America, Australia and Africa, and the fourth grouping in the Caribbean. Based on the antigens present in their chemical composition there are antigenic relationships between different serotypes, but under natural conditions, each serological type confers protection to animals only against the homologous virus type. The distribution of serotypes 1, 2, 4, 8, and 16 are currently found within Europe. BTV is related antigenically and with some serotypes of the deer epizootic haemorrhagic disease virus [6].

Ecology

To BTV infection are susceptible most domestic and wild ruminants, including, sheep, goats, cattle, water buffalo, African buffalo (*Syncerus caffer*), bison, deer, bighorn sheep, elk, most species of African antelope, and other *Artiodactyla* such as camels. Studies made in recent years suggested that BTV can infect carnivorous domestic or wild species, even lions, cats and dogs. Because in many cases a BTV infection not always resulted with a clinical disease, the reservoirs of viruses can be ruminants with subclinical infections, especially cattle, but not excluded goats or dromedaries, while sheep most of cases show clinical signs, developing clinical disease. There are differences in race-related receptivity, the breeds being more receptive than indigenous and age-related differences, which inexplicably vary from one epidemic to another, but younger animals are usually more receptive. The exposure of animals receptive to solar radiation for a long time, as well as any form of stress, are important factors in outbreak of the disease. Also, BTV infections have been reported in at least 80 species of wild ruminants. The main sources of infection are mainly sick or diseased sheep, as well as bovine or some contaminated wild ruminant species in which blue-tongue disease develops inappropriately or with minimal clinical signs. In diseased animals, the virus is found in the blood, lymph nodes and spleen. Vertical transmission of the disease is possible and trans placental transmission to the sheep and the cow has been reported. If infection occurs during the first part of pregnancy, embryo death occurs, and when done in the middle of gestation, teratogenic effects occur in embryos. When embryo infection occurs shortly before parturition, the newborn becomes virulent after calving. The most important role in the transmission of the disease is the hematophagous insects of the genus *Culicoides*. More than 1000 species of *Culicoides* have been described throughout the world, but only a limited number of species are involved in the transmission of the blue tongue virus. In the United States, *Culicoides variipennis* is the main vector, with the exception of the South East and Central America where the virus is transmitted via the *Culicoides insignis* species. Culicoids are active only at temperatures between 13° C and 35°C and feed on the animals only at night. Birds of these insects are exposed to the animals kept in open shelters or those found on the pasture on the pasture. The virus, with the blood in the insect body, responds, and after 10-15 days it locates in the salivary glands [7, 8]. Each insect so infected remains infectious for the rest of the life. Although bluetongue disease was already recognized in South Africa in the early 19th century, a comprehensive description of the disease was not published until the first decade of the 20th century. In 1906 Arnold Theiler showed that bluetongue was caused by a filterable agent. He also created the first bluetongue vaccine, which was developed from an attenuated BTV strain. For many decades bluetongue was thought to be confined to Africa. The first confirmed outbreak outside of Africa occurred in Cyprus in 1943.

Epidemiology

Bluetongue disease develops endemically in tropical areas where hematopoietic insects remain active throughout the year. In temperate climates, the disease has a seasonal character and evolves especially towards the end of summer, when the density of culicoids reaches the maximum [9, 10]. In areas where the disease develops epidemically, the incidence of clinical expression in exposed sheep varieties varies depending on the density of the insect population, the virulence of the strain of the virus, the immune status of the sheep, the severity of the clinical signs, etc. Another possibility of transmitting these diseases from one region to another, or from one country to another is the strong air currents that can drive the movement of the culicoids. It is believed that the occurrence of BT in 1977 in Cyprus was caused by the infected insects brought by the air currents

across the sea. The rate of morbidity and mortality varies widely and depends on certain factors. When disease first appears in a herd of sheep, morbidity falls between 50-75% and mortality between 20-50%. In disease episodes that have evolved in Cyprus and Spain, mortality rates of about 70% have been reported, while in the US it is between 0-14%. In South Africa were reported mortality rates ranging from 2-30%. Regardless of the geographical area, the mortality rate increases when a strain of new virus enters the livestock. Cattle rarely produce severe clinical signs of bluetongue, although the virus can be isolated even after 81 days of infection. Bluetongue disease develops endemically in tropical areas where hemophagocytic insects remain active throughout the year [11-13]. In temperate climates, the disease has a seasonal character and evolves especially towards the end of summer, when the density of culicoids reach maximum. The severe progression of this disease in the episodes that occurred in Cyprus and the Iberian Peninsula in the 1940s and 1950s has made the disease of the blue tongue now included in the A list of the OIE. When appearing in a herd, official quarantine measures of I degree are officially declared and instituted.

Pathogenesis

Inoculation of BTV in the organism of susceptible animals is usually performed only by the hematopoietic insects of the genus *Culicoides*. They are also contaminated by the sucking of the blood from the diseased animals. About 5-10 days after the contaminated bite, the BTV is located in the salivary glands of the insect, and it remains infected for its entire life, transmitting the virus through the saliva whenever the insect squeezes a receptive animal. Approximately 3 days after infestation, a brief period of viremia is detected, and after 6-7 days of treatment, a second period of viremia accompanied by fever and leucopenia. With the occurrence of interferon and specific antibodies, circulation of the circulating blood virus decreases, but it is localized at the vascular endothelium level, where it replicates and causes epithelial ischemia in which it is localized. The virus also replicates in the Malpighi layer cells and the decomposing layer of the tongue, lips, esophagus, rumen and skin. The lesion distribution appears to be influenced by the mechanical stress and lower temperature of these tissues or organs compared to the rest of the body tissues [14, 15]. The presence of the blue-tongue virus within the erythrocytes suggests that the virus replicates mainly in the haematogenous bone marrow in the stem cells employed on the erythrocyte line and explains the existence of the virus in the infected animal's organism in the presence of specific antibodies. The epithelial cells in which the virus replicated suffer a degeneration process (sometimes even necrosis), detach and favor the appearance of characteristic erosions or ulcers. In the case of pregnant animals, the virus crosses the placenta and, depending on the stage of gestation, causes abortion or congenital abnormalities of the fetus, located mainly in the central nervous system. The presence of blue-tongue virus in semen of rams or bulls is often accompanied by structural abnormalities of sperm, which demonstrates that the virus also replicates in vascular endothelium at the testis. In BTV infection of the white-tailed deer there is evidence of endothelial cell degeneration as a result of virus replication at this level accompanied by necrosis and hemorrhage, coagulation changes with the occurrence of bleeding diathesis syndrome, making it very difficult to differentiate BT of the disease determined in this species by the epidemic deer disease of the deer [16-18].

The disease is not transmitted from one animal to another or to humans, but it causes economic damage due to the costs of disease eradication, including: compensation for killed animals, expenditure related to disease eradication activities (personnel, materials, equipment, etc.) expenditure on land rendering, insect control and disinfection activities. In countries where disease is developing registered large economic losses due to blocking the exports of cows and sheep.

Clinical evolution

Sheep are the most susceptible to BTV infection, which may be subclinical or result in mild to severe clinical signs. The incubation period is 5–20 days, and all signs usually develop within a month [11]. In cattle, goats and wild ruminants infection is usually asymptomatic despite high virus levels in blood. Red deer are an exception,

and in them the disease may be as acute as in sheep. The mortality rate is normally low, but it is high in susceptible breeds of sheep. Signs of illness can include fever, excessive salivation, depression; dyspnea and panting; nasal discharge that becomes mucopurulent and crusts around the nostrils; hyperemia, congestion, and edema of the head and facial tissues; and ulceration and necrosis of the oral mucosa. The coronary bands of the hooves may become hyperemic and can result in lameness due to the pain. Pregnant ewes infected with BTV may abort their fetuses or give birth to malformed lambs. Additional clinical signs may be torticollis, pneumonia, emaciation, and conjunctivitis. Cattle and goats have subclinical infections, but in clinical cases, the presentation is similar to that in sheep. If present, signs of illness may include facial edema, oral inflammation with vesicles or ulcers in the mouth, excess salivation, nasal discharge, crusting around the muzzle, edema of the distal limbs, hyperemia of the coronary band with lameness, udder and teat lesions, decreased milk production, and abortions or births with central nervous system lesions resulting in "dummy" lambs. Specifically, cattle can have skin lesions varying in severity, including pododermatitis that leads to thickening, cracking, and sloughing [19-21]. In the wild life, cervids can present with clinical signs depending on the serotype; however, subclinical infections are most common. White-tailed deer tend to have the following signs in addition to those presented in sheep: severe fever, depression, anorexia, and loss of normal fear responses. Pronghorn may suddenly die or have prolonged sickness. There is little information on other cervid species.

Bluetongue in Europe and in Romania

Since 1998, probably caused by climatic changes, BTV spread northwards into the Mediterranean Basin, where BTV serotypes 1, 2, 4, 9 and 16 have been identified [22-24]. In the summer of 2006, for the first time, the BTV has exceeded latitude 50° N and BT outbreaks caused by BTV serotype 8 appeared in North-Western Europe: in Netherlands, Belgium, Germany, France, and Luxembourg [5, 25, 26]. In 2007-2008, the BT has worsened and BTV8 spread to the other regions of Europe, the number of outbreaks increasing rapidly (about 50000 cases of BT were reported). Unfortunately, two new BTV serotypes, BTV-1 and BTV-6, were detected [5]. But implementation of BT compulsory vaccination schemes in Europe in spring 2008 resulted in reduction of BTV-8 cases from about 50000 in 2007-2008 to 350 in 2009 and 19 in 2010. The last case of BT caused by BTV-8 was reported in May 2011 in north Sardinia (Italy) [26]. From the endemic areas, since 1999 there have been reports of outbreaks of BT in Greece, Italy, France (Corsica), Spain and Portugal. Cases also occurred in Europe in Albania, Bosnia and Herzegovia, Bulgaria, Cyprus, Croatia, Former Yug. Republic of Macedonia, Serbia and Montenegro and Turkey reported on World Organization for Animal Health (OIE) website [27].

In August 2006 Dutch, Belgian and German authorities have officially notified the World Organization for Animal Health (OIE) of the occurrence of an outbreak of Bluetongue in sheep on their territory. Dutch on August, the outbreak affected a farm in the province of Limburg. Bluetongue was confirmed by laboratory investigations. Dutch, Belgian and German authorities have taken classical disease control measures such as zoning and movement control and continuous surveillance within the country.

In September 2007 the first ever case of bluetongue disease has been reported in the UK, in a cow at a farm near Ipswich, Suffolk. From August 2006 till September 2007, a number of 1,833 farms from Germany had reported the presence of the infection. In a week in September 2007, the number of farms reporting infections has grown to 5,686. The number of deaths is estimated around 15,000 sheep. The origin of the infection was not clear but it was one of the diseases which invaded Northern Europe probably as a consequence of global warming. Bluetongue has also been found in Belgium, France, the Netherlands and Luxembourg. The United Kingdom Department for Environment Food and Rural Affairs says the virus could spread from Northern Europe to the UK, but was unlikely. In 2007, the first case of bluetongue in the Czech Republic was detected in one bull near Cheb at the Czech-German border.

In October 2007 bluetongue had become a serious threat in Scandinavia and Switzerland and the first outbreak in Denmark was reported. In autumn 2008, several cases were reported in the southern Swedish provinces of Småland, Halland, and

Skåne, as well as in areas of the Netherlands bordering Germany, prompting veterinary authorities in Germany to intensify controls. Norway saw its first finding in February 2009, when cows at two farms in Vest-Agder in the south of Norway showed an immune response to bluetongue. Norway has been declared free of the disease in 2011. In April 2008 in European Commission, in Directorate D-Animal Health and Welfare in a working group on bluetongue transmission and surveillance were presented the preliminary results in investigation of the possibility of trans placental and persistent infection with BTV. All these debates and results started from a case in Northern Ireland with 54 calves from 20 farms were trans-placental infected in proportion of 1⁄3 with BTV-8. The same way of transmission was observed too in farms from Belgium, Netherlands, Slovakia, but in Spain results proved did not identify any case of trans-placental transmission with BTV-1 in calves. The conclusions of the report indicated that trans-placental transmission in cattle is proven for BTV-8 but so far no proof yet of trans-placental transmission in sheep. Immune-competent and immune-tolerant calves are born from infected dams. The preliminary conclusions indicate that trans-placental transmission ob BTV-8 occurs and it does so frequently but it has to be confirmed if this is the major means of over-wintering in Northern Europe. There were multiple outbreaks of BT into southern Europe between 1998 and 2005 and in 2006 BTV-8 was reported in northern Europe. BTV-8 successfully overwintered in 2006 and 2007 and spread widely during 2006, 2007 and 2008. It spread to the UK in 2007, with vaccine being used in disease control in 2008 in the UK and Europe more widely. In Scotland, compulsory vaccination of cattle and sheep was implemented in 2008. Recently researches had implicated the *C. obsoletus* complex, including *C. dewulifi*, as competent vectors for transmission of BTV-8. The recent migration of *C. imicola* into the Mediterranean region, historically only a resident of Africa, tested positive for BTV-8, attributing to serotype 8 transmissions. The introduction of BTV-8 into Europe has resulted in significant economic losses due to animal morbidity/mortality and trade restrictions. In 2009 BTV-1 and BTV-8 were isolated from outbreaks in west and south-west part of Europe. In 2010 were registered isolated outbreaks of BT with BTV-1, BTV-2, BTV-4 and BTV-8 in south and south-west part of Europe. In 2011 only 9 outbreaks of BT were registered in south and 2 in south-west part of Europe, with the same serotypes, plus BTV-9 [28]. The year 2012 BT evolution registered a few outbreaks in the same areas, with BTV-1, BTV-2, BTV-4 and BTV-9 serotypes, but 2013 evolution was in south, with BTV-1, BTV-2 and BTV-4 serotypes. Blue-tongue disease has never evolved in Romania. Serological disease surveillance has been introduced since 2000, and entomological surveillance was introduced in 2005, when vectors belonging to the species *C. obsoletus*, *C. pulicaris*, *C. nubeculosus* were identified. The free vector period usually begins in November and ends at the end of April [29, 30-32]. In September 2008, a part of Romania's territory was integrated into a surveillance zone due to the outbreak of bluetongue (BLV 8) on the territory of Hungary. In April 2009, Romania recorded the results of the disease surveillance action carried out in the surveillance zone and requested the lifting of the restriction measures [33]. In 2014, as a result of BTV-4 in Greece and Bulgaria, alerts were daily alerted to other countries for the purpose of informing and stepping up surveillance and measures to control the movement of animals. The bluetongue disease was confirmed for the first time in Romania country on 22 August, following laboratory tests [34]. In Buzău County, 28 outbreaks were confirmed in the localities of Săruleşti, Bisoca and Beceni, and the disease affected 73 cattle and 5 sheep. In Europe, in 2014, blue-tongue disease was confirmed in Italy, Spain, France, Portugal, Greece and Bulgaria. Because this disease has not been reported to us in the country, and because more cattle in Buzau County have been diagnosed with blue-tongue disease, which does not affect humans but produces economic damage, ANSVSA announced that it informed the EU and the members of the World Organization for Animal Health on the occurrence of this disease in Romania. The disease had an expansion in our country between August 23 and October 28, 2014, affecting several counties, several animals, new cases of disease being confirmed by laboratory analysis. Until December 3, 2014, outbreaks of bluetongue (BTV- 4) were confirmed in 34 of the 42 counties in our country. For our country, the mortality in bluetongue disease was not so high, affecting 6,691 animals,

of which 2,849 died, but if we refer to the number of receptive animals of about 16 million it is considered to have been a percentage. Romania has declared the free vector of blue vectors since December 2, when the vector involved in the transmission of the disease is no longer active in our country's climate, with the restrictive measures imposed by our legislation and the European Commission being lifted, after which the outbreaks of the disease gradually extinguished. On September 4, 2015, the National Sanitary Veterinary and Food Safety Authority (ANSVSA) informed that it had diagnosed Bluetongue in Bobuleşti, Botoşani County, near the Prut River and the border with the Republic of Moldova. Subsequently, infected animals were also identified in two other localities in the same areas, namely Badiuţi and Ştefăneşti. Until that date, the disease was confirmed in 18 cattle from 18 holdings, as follows: in the locality of Bobuleşti 12 cattle from 12 non-commercial farms, in the locality Badiuţi 4 cattle from 4 non-commercial farms, respectively in Ştefăneşti 2 cattle from 2 non-commercial holdings. According to the cited source, the 18 infected bovine animals returned after symptomatic treatment, with no "mortality". In September 2015, 157 commercial cattle holdings have been registered for voluntary vaccination against bluetongue in 32 counties, the total number of treated animals being 82,173 cattle and the need for vaccine doses of 167,961 [35]. In February 2016, the French reported 61 cases of blue-tongue disease to 61 cattle (including one death) and six cases of sheep. The bluetongue outbreaks detected in France were largely caused by the BTV-8 serotype, while in other European countries; the disease was caused by the BTV-4 serotype. Specialists in the field concluded that Romania can control and eliminate blue-tongue disease only by vaccinating all ruminant animals for a period of three years. Article 8 of Commission Regulation (EC) No 1266/2007 foresees that exemptions from exit ban are to be based on risk mitigating measures presented in Annex III of the regulation or on any other appropriate animal health guarantees based on a positive outcome of a risk assessment agreed between the competent authority of the place of origin and approved by the competent authority of the place of destination [36-38]. Currently there are such agreements on the movement of live animals between: Austria and Slovenia of 2017, France and Italy of 2015, France and Luxembourg of 2015, France and Spain of 2013, 2015, 2016 and 2017, Hungary and Austria of 2016, Italy and Austria of 2016 and 2018, Italy and Hungary of 2016, Italy and Spain of 2012, Spain and Portugal of 2014. The BT affected zones in Europe in accordance with Article 2 (d) of Commission Regulation (EC) No 1266/2007 established the restricted zones for a specific bluetongue serotype or combination of serotypes and the "lower risk areas" which are demarcated in accordance with Article 7, paragraph 2a of the Regulation (Table 1 and Figure 1). The European Commission established in the last three years registered situations the updated bluetongue outbreaks in Europe (Table 2). From these data it can be seen that in most countries the BT situation is under control, excepting France and Italy, with many BT outbreaks in 2016 and 2017 [39-41]. All farmers, practitioners and other relevant stakeholders should be vigilant and ensure that they are fully aware of the presenting clinical signs of bluetongue in both cattle and sheep, and that they report any suspicion of disease to their Veterinary Practitioner or Regional Veterinary Office (RVO) without delay, single condition to help stave off this disease [42, 43].

Table 1. The restricted zones for a specific bluetongue serotype or combination of serotypes in accordance with Article 2 (d) of Commission Regulation (EC) No 1266/2007 and the "lower risk areas" which are demarcated in accordance with Article 7, paragraph 2a of the Regulation

Restricted zone	Serotype(s)	Member State	Territories	Date last update
Zone Y	8, 4	FR (France)	Mainland teritories	1.01.2018
Zone G	1, 2, 4, 16	IT (Italy)	Calabria, Campania	12.01.2018
Zone H	Not specified	MT (Malta)	Whole territory	23.05.2007
Zone I	1, 4	HR(Croatia)	Lastovo island	22.09.2015
Zone I	1, 4	ES (Spain)	Andalucia, Toledo	4.02.2016
Zone I	1, 4	IT (Italy)	Toscana, Liguria, Lazio	12.01.2018
Zone J	1	PT (Portugal)	Whole teritory	7.11.2013
Zone T	1, 2, 4, 8, 16	FR (France)	Haute Corse, Corse du Sud	16.04.2009
Zone X	4, 16	EL (Greece)	Dodekanisa, Samos islands	6.03.2013
Zone A_3	4	EL (Greece)	Whole territory	
Zone A_3	4	BG (Bulgaria)	Whole territory	13.08.2014
Zone A_3	4	RO (Romania)	Whole territory	10.10.2014
Zone A_3	4	HU (Hungary)	Whole territory	23.11.2015
Zone A_3	4	SK (Slovakia)	Whole territory	23.11.2014
Zone A_3	4	HR (Croatia)	Whole territory	25.11.2014
Zone A_3	4	ES (Spain)	Toledo, Castilla, Salamanca	4.02.2016
Zone A_3	4	AT (Austria)	Carinthia	25.01.2018
Zone A_3	4	SI (Slovenia)	Whole territory	26.08.2016
Zone A_3	4	IT (Italy)	Veneto, Liguria, Lombardia	12.01.2018
Zone A_3	1, 4, 8, 16	EL (Greece)	The island Lesbos	8.09.2009
Zone A_5	1, 3, 4, 16	IT (Italy)	Sicilia	4.12.2017
Zone A_6	1, 4, 16	IT (Italy)	Puglia, Sicilia, Lazio, Umbria, Molise	12.12.2017
Zone A_7	4, 16, 8	CY (Cyprus)	Whole territory	20.09.2016
Zone A_8	16	EL (Greece)	The island of Kos	13.11.2017

Table 2. Outbreaks of bluetongue in Europe since 2015
(Home page address:http://ec.europa.eu/food/animals-diseases/control- measures/bluetongue en)

Country	2015	2016	2017
Austria	4	3	0
Bosnia & Herzegovinia	0	4	0
Croatia	22	52	3
Cyprus	0	171	0
France	143	1294	1325
Hungary	37	0	0
Greece	2	2	3
Italy	323	990	1425
Montenegro	1	0	0
Portugal	27	27	2
Romania	30	0	0
Republic of Serbia	0	150	0
Slovenia	1	27	0
Spain	19	20	3
Switzerland	0	0	2

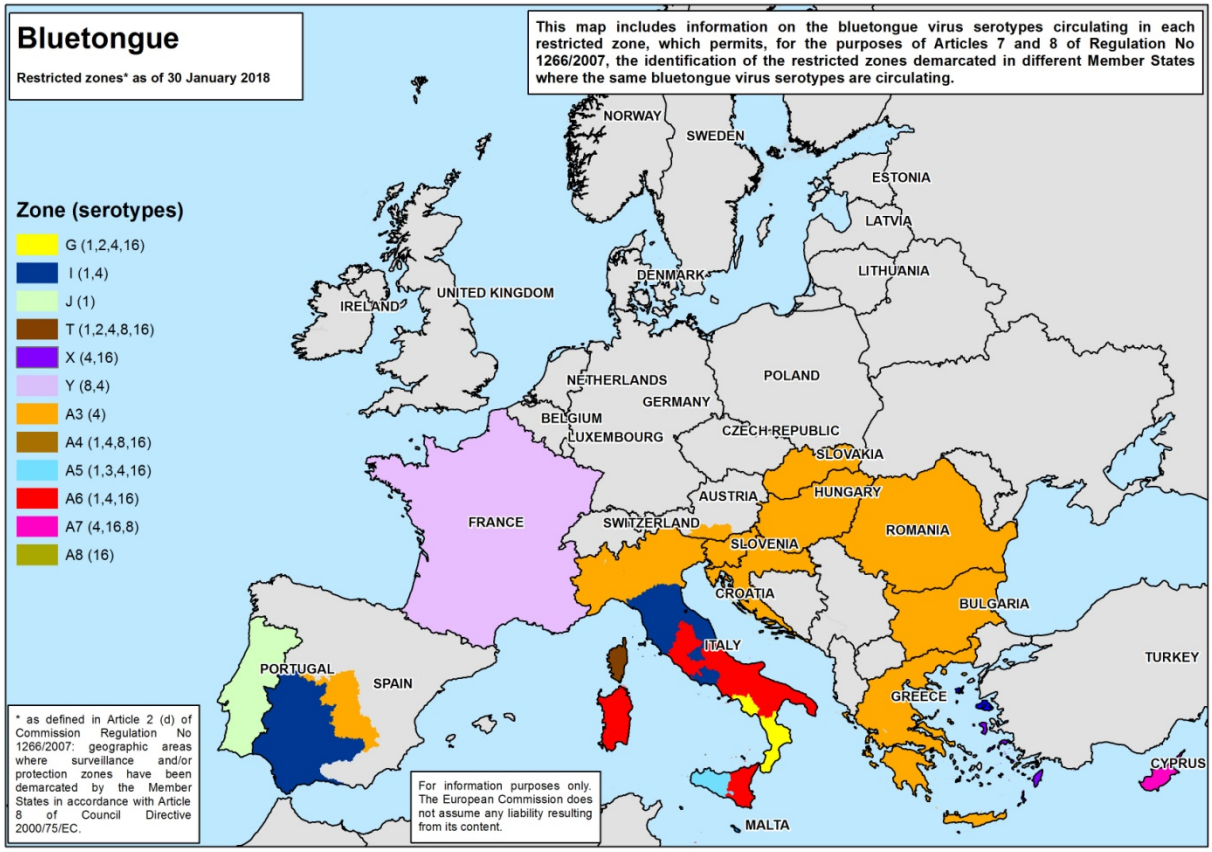

Figure 1. The BTV serotypes in Europe (European Commission website, 2017 (Home page address:http://ec.europa.eu/food/animals-diseases/control- measures/bluetongue en).

Conclusions

BTV is an endemic etiologic agent in many tropical, sub-tropical and temperate regions of the world (America, Australia, Africa and some regions of Asia), between latitudes 40° S and 53° N, during times of the year that are optimal for vector activity, in Europe in the past had only sporadic incursions, involving in a single virus serotype. Probably due to climatic changes, since 1998 BTV developed in Europe many serotypes and in 2006, for the first time crossed latitude 50° N and BT situation changed for the worse, invading new areas, with rapidly increased number of outbreaks. All these outbreaks ended up with a significant economic impact, mainly due to the disease effect on animals (morbidity, mortality, reproductive failure, reduction in milk yields and weight gain) and, most of all, to the disruption of international trade of animals and animal products. Neither our country has made an exception in BT outbreaks, in 2014, for the first time this disease appeared in Romania, correlated with exceptional climatic conditions, with heavy rains caused by different atmospheric fronts, have caused the appearance and confirmation of the bluetongue disease. The vector involved in transmitting blue-tongue disease is *Culicoides*, a mosquito that does not live in our country but has occasionally been observed in entomologists' pitfalls since 2005, when vectors belonging to *C. obsoletus*, *C. pulicaris*, *C. nubeculosus*. Such a hematophagic insect can travel up to 1.5 to 2 kilometers per day in an area, and under certain weather conditions (winds that predominate in a certain direction and period under favorable conditions) insects can be carried away far more large, which can reach up to 200 kilometers per day, making possible the rapid spread of the disease on the territory of our country. Control and eradication of BT by conventional methods such as movement restrictions or stamping out is hardly possible, due to asymptomatic infections, prolonged viremia in cattle, and virus persistence in the midge

population. Therefore, vaccination with effective vaccines against the right serotype is the preferred method to control BT [44, 45]. The aim of BT vaccination differs for different field situations. Vaccination could manage at prevention of morbidity and mortality in the vaccinated population, reduction of virus spread in the field, and eradication of BTV to allow safe movement between BT-affected and BT-free zones. The efficacy of BT vaccines is important. Following a rapid stop of virus transmission after an incursion, a quick onset of immunity is more important than duration of immunity. The veterinary vaccines should be cheap, because of the often low economic value of livestock, such as sheep and goats. In the developed world, with a high density of high-value breeds of cattle, vaccination costs can be higher, especially when aiming at a BT-free status. In poor, developing countries, a low vaccine price is very important to be accessible to all farmers to immunize livestock. Finally, in multiple serotype situations, vaccines should protect against all circulating serotypes in the area. The epidemiological inquiry confirms the more cases of BTV infection among domestic ruminants in the regions where there are wild animals, mainly from *Cervus* family. It can be concluded that wild ungulates due to the long-term carrier state may act as a reservoir for BTV and play an important role in its transmission.

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