„Regional action on animal disease eradication in the Western Balkans”
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PROJECT ACTIVITY:

A3.7 – DESIGN A STRATEGY FOR VACCINATION. THE STRATEGY MUST INCLUDE TOOLS THAT MAKES IT POSSIBLE TO ESTIMATE THE EFFICIENCY OF EXISTING VACCINATION PROGRAMS IN THE BENEFICIARY COUNTRIES. THE STRATEGY NEEDS TO CONTAIN ALL ASPECTS OF A VACCINATION PROGRAM, SUCH AS: DISEASE, SUSCEPTIBLE ANIMALS, VACCINE(S) AVAILABLE, HOW TO USE THE VACCINE (IS IT APPLICABLE ALL YEAR ROUND), HOW IS THE EFFECT OF THE VACCINATION PROGRAM MEASURES, PROCEDURES TO REASSURE THAT THE PROGRAM WILL BE CHANGED IF IT IS NOT WORKING AS INTENDED, HOW TO BUY THE VACCINE AND HOW TO GET ACCESS TO FUNDING OF THE VACCINE

and

A3.8 - DEVELOP A STRATEGY OF HOW TO DEAL WITH EMERGING DISEASES

COHERENT STRATEGY FOR BLUETONGUE CONTROL INCLUDING VACCINATION IN THE WESTERN BALKAN REGION

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IMPORTANT EPIDEMIOLOGICAL INFORMATION

INTRODUCTION

Bluetongue (BT) is a notifiable disease of wild and domestic ruminants caused by Bluetongue virus (BTV), an *Orbivirus* of the *Reoviridae* family. Sheep, cattle, deer, goats, camelids (camels, llamas, alpacas, guanaco and vicuña) and many other species could be infected but, with rare exceptions, disease is generally observed in ruminants only. Sheep are the most susceptible species and sudden death is common. Severe clinical signs could be observed in cattle, goats, deer and other ruminant species. The severity of the disease can depend on many factors including breed, host health status, BTV strain/serotype. Clinical diagnosis can be difficult as clinical signs are not specific and similar to other notifiable diseases. In many cases, animals can appear healthy when infected allowing the infection to silently spread. There are currently 27 officially recognised BTV serotypes, however many other BTV strains have been described although not officially recognised yet. BTV is mainly spread by adult infected midges biting an infected and viraemic animal. Vector-free transmission has been hypothesised or demonstrated for BTV-25, BTV-26, BTV-27 and some other new BTV strains recently described. For this reason, they are called atypical to be distinguished from the typical or traditional serotypes. For the traditional serotypes, their possible spread through direct contact between live animals is a rare event with little epidemiological importance. Conversely, for these vector dependent BTV serotypes, the time of year, i.e. whether during the active vector season (normally March-November), and meteorological conditions, i.e. temperature and wind direction, and the proximity and density of neighbouring farms, are significant factors in their potential incursion and spread. They are not spread through carcasses or fomites, such as on vehicles. With regards to vehicles, though, attention should be paid on those carrying infected midges which could be a potential route of spread over large distances.

Transmission of BTV is definitely influenced by environment, weather pattern and biology of vector species. Climate changes appear to have increased both the distribution and abundance of vector populations in the European region and have been suggested to have influenced the vector capability. As a result, BTV can now be transmitted by “novel vectors” (e.g., *C. obsoletus* and *C. pulicaris*) that are abundant across Eastern, Central and Northern Europe. They have supported and support the persistence and spread of many of the serotypes associated with the initial outbreaks across much of Eastern, Southern and Central Europe. Regarding the virus-vector relationship, it has also been supposed that within distinct ecosystems and in separate geographic regions, different vector species maintain several distinct virus strains (topotypes) in a quite stable relationship despite extensive and ongoing trade and movement of ruminants. Due to the geographical variation reliant on this vector-virus association, several distinct, but partially overlapping, epidemiological systems (episystems) have been formed in most regions with significant BTV infection, including Europe, North America and Asia.

EASTERN MEDITERRANEAN EPISYSTEM

In the Eastern Mediterranean, a bluetongue episystem has been known since the first half of the 20th century with sporadic spillovers in Cyprus and Greece. The numerous BTV incursions and clinical outbreaks which have been recording in the Balkans starting from the end of 20th century are nothing else than the evolution of the historic Middle East episystem. In this Region, most of the BTV spread have been associated either to animal movements or to the spread of vectors by proximity. The main *Culicoides* species acting as vectors in this area belong mostly to the Obsoletus and Pulicaris Complexes although *C. dewulfi*, and *C. chiopterus* have also been found.

Due to the setting of new and favourable environmental and climatic conditions, **it is expected that the incursions from the Eastern corridor and, consequently, BTV incidence will increase in the next coming years with dramatic losses on livestock industry and severe socio-economic consequences.**

BACKGROUND ON BTV CONTROL STRATEGIES

INTRODUCTION

Europe was not fully prepared to tackle the magnitude of the bluetongue epidemic at the turn of the 20th century. In the European Union, the strategy of bluetongue control was essentially based on stamping-out. Vaccination was considered as a complementary measure to stamping out, to control direct losses and to limit the spread of infection. Also in the Balkans, after the first BTV incursion in 1999, most of the actions were not preventive but only in response to outbreaks. The situation rapidly modified at the end of 2000 with Directive 2000/75/EC as the inadequacy of the stamping out to control vector borne diseases became evident. It and its subsequent amendments set out detailed measures to control and eradicate bluetongue disease, outlining procedures to be adopted in the event of an outbreak. The basic control strategy is based **on strict movement controls** of the susceptible animals from infected zones and **vaccination**, initially limited to sheep but then extended to all susceptible domestic ruminants that could be exposed in the protection zones. Intensive clinical, serological and entomological surveillance is recommended to define the areas that are subjected to movement restrictions. Really different was instead the reaction by Balkan countries to the 2014 BTV-4 incursion. In line with the EU legislation, each country put in place its own control measures according to the disease impact, livestock sector organization and financial resources. Although mitigating the BTV impact in the countries, these measures were not able to impede BTV-4 to re-emerge and spread. Since BTV dissemination in the Balkan Peninsula is largely due to the active short distance flights of infected *Culicoides*, it is expected that measures mainly limited on movement control are inefficient if not associated with other preventive measures such as vaccination. In the absence of vaccination, in fact, the virus circulation can persist for several years at low level, creating the appropriate conditions for BTV endemization and re-emergence. The mass vaccination carried out in some countries was capable to protect livestock at national level, yet did not influenced and prevent the re-emergence and spread of 2014 BTV-4 strain in the region in 2020. Consequently, in the light of the above, it appears clear that **to optimise the effort and improve the results in controlling Bluetongue, it is necessary to think of a common and**

coordinated strategy which involves all Balkan countries and includes both preventive and control measures.

PRELIMINARY GENERAL REMARKS

In Europe, the Council Directive 2000/75/EC lays down specific provisions for the **control and eradication of Bluetongue**. The measures to control and eradicate the disease include vector control, (use of insecticides in the animal premises and in the areas where these insects live, insect repellents onto animals, mosquito nets, etc.), restriction to movements of live ruminants from affected areas to non-infected regions where the vector is present and the use of vaccines. Directive 2000/75/EC will be replaced by Regulation (EU) 2016/429 from 20 April 2021.

The strategy to control the risk of BTV disease should aim to (i) reduce the likelihood of outbreaks by reacting to prevent disease incursion, (ii) early detect and rapidly apply appropriate control measures if incursion does occur, (iii) implement surveillance, contingency planning to reduce the impact of such an incursion.

Within this perspective, **control of animal movements, surveillance and vaccination** become then fundamental for the success of the BTV prevention and control strategy. Their efficacy will definitely improve if **information systems capable of guaranteeing the collection and rapid exchange of data** on (i) animal population structure, (ii) animal movements, (iii) disease presence (no. of outbreaks, no. of cases, etc…), (iv) vaccination status, **are in place**. Data collection is essential to evaluate the health status of the population, recognize a free status, support risk analysis process and evaluate the efficacy of control measures.

A general overview of these three main strategic activities is here provided in order to get information about their pro and cons.

a) Surveillance

Surveillance is used to delineate with precision those areas in which the virus is circulating and, consequently, to enforce the appropriate animal movement restrictions. It can also provide the data required to assess the risk associated with animal movement and trade. In this regards, considering that BT is a vector borne disease, monitoring the presence and abundance of potential vectors is also important. Bluetongue surveillance programs should then include entomological surveillance, clinical (or passive) and active surveillance.

In areas with high density of livestock and susceptible animals (i.e. sheep), clinical surveillance represents a very efficient early detection system. Conversely, in regions densely populated by species which don’t show clinical signs after BTV infection (i.e. bovine) and/or where vaccination campaigns are in place, other more efficient system should be implemented for early detection. In these cases, it would be better to do active case finding in the animal population.

Active surveillance is based on the periodical serological testing of unvaccinated and BTV negative sentinel animals, preferably cattle, that are uniformly scattered throughout a region in a grid of uniform cells. These sentinel animals should be sampled at least once a month during the vector

season, if known. In the absence of such information or in areas where vectors fly all year round, the animals should be tested at least once a month throughout the year. Laboratory tests by demonstrating or not demonstrating BTV circulation represent a crucial step. These data add an important dimension, through routine reporting of positive diagnoses to local and national animal health authorities. By including some asymptomatic infections, they further improve any epidemiological estimate; additionally, information on particular serotype or viral strains may be fundamental for the application of preventive measures.

The serological surveillance aims to:

- Monitor the BTV circulation and, in case, demonstrate the absence of viral circulation. According to the EU regulation, if the absence is demonstrated for at least two vector seasons, the Member country may request to be declared free. In line with the new EU legislation as described in the delegated act of the Animal Health Law (Regulation 689/2020), the surveillance should be able to demonstrate, with a 95 % level of confidence, that the prevalence rate of the disease is lower than 1 %.

- Identify a possible new incursion or re-occurrence of infection with those BTV-strains which haven't been reported in the previous 2 years. In line with the new EU legislation as described in the delegated act of the Animal Health Law (Regulation 689/2020), this surveillance should be able to detect, with a 95 % level of confidence, the infection in the targeted animal population at a prevalence rate of at least 5 %.

The minimum geographical unit of reference for monitoring the bluetongue can be represented by a grid of around 45 × 45 km (2,025 km^2). Reducing the size of the grid will increase the sensitivity of the system, and then, the probability to early detect the virus in case of its introduction. Moreover, smaller is the size of the grid, more precisely will be defined the areas of actual virus circulation and restrictions to animal movements.

To detect a prevalence rate of 1% or higher, at least 300 randomly selected animals per grid should be tested while, if the objective is to detect a prevalence rate of 5%, 58 randomly selected animals should be tested.

Sentinel animal should be tested by c-ELISA and positive results should be confirmed by serum-neutralization assay.

As alternative to the serological surveillance, to detect the incursion of new serotypes (serotypes even not included in the vaccine), the animals under the programme could be selected using a stratified random sampling approach and then tested by RT-PCR. Stratification should be done on a geographical base by municipality or at least by province. The target population to be tested using RT-PCR can include slaughtered animals and animals tested in the framework of eradication or surveillance programme for other diseases. For each geographical unit, 87 animals should be selected and tested each month. This will guarantee the same sensitivity of the surveillance plan based on 58 sentinel animals monthly bled. In comparison to the number of animals required for the serological surveillance, that required for virological surveillance depends on the duration of

viremia detectable by RT-PCR. This approach does not require frequent visits to the farm by the veterinarian, with a clear advantages related to both financial and organizational aspects. The abattoir system, however, needs to correlate sampled animals with the farm of origin and stratify the sampling according to the origin of the animals.

Entomological surveillance is important to monitor the abundance and dynamic (seasonality) of vector population in certain areas. Based on the use of black-light traps which should operate from sunset to sunrise, it (i) gives important information on the distribution map of *Culicoides* spp for the countries, (ii) elucidates the seasonal dynamics of all vector *Culicoides* in order to identify zones that are seasonally free of these vectors, and (iii) updates the *Culicoides* species responsible of BTV transmission during outbreaks. The traps should be positioned in fixed location and operate on a weekly base.

b) Vaccines and vaccination

BT vaccines may be used for different purposes or strategies, depending on the epidemiological situation of the affected area and desired policy objectives. The main objectives of BT vaccination strategies are to (i) prevent clinical disease, (ii) reduce the spread of BTV, (iii) reduce BTV circulation to enable eradication of BT from the country or the region, and (iv) permit the safe movement of susceptible animals between BT-affected and BT-free zones. These goals have guided BT vaccination campaigns since the incursion of BTV into Europe, and these efforts have allowed the eradication of serotypes 1, 2 and 8 in some regions. It is therefore commonly accepted that vaccines can help limit the spread of BTV. Inactivated, whole-virus and live, attenuated virus vaccines (LAVs) are the only non-replicating and replicating BT vaccines available in the market.

i. Live attenuated virus vaccines (LAV)

Because of their ability to replicate in the vaccinated host, LAVs usually stimulate a strong immune response. They are inexpensive and are capable of stimulating protective immunity after a single inoculation. They have proven to be effective in preventing clinical BT disease in the areas where they are used. Several documented or potential drawbacks are, however, attributed to BT LAVs. These include under-attenuation, which in sheep may sometimes cause severe clinical signs. Adverse responses to LAV vaccination can include depressed milk production in lactating sheep, temporary infertility in rams and ewes, and abortion/embryonic death and teratogenesis in offspring when these vaccines are used in the first third of pregnancy. Besides, LAVs may induce viremia with a virus titer sufficient for infecting midges and being spread in the environment. Case of reassortment due to vaccine virus has been described in Europe, the frequency and significance of these events however remain uncertain. Despite the risk and shortcomings of LAVs, these types of vaccines are used in many BT endemic parts of the world since LAVs are effective and cheap, whereas the adverse reactions are limited in local breeds. It should be kept in mind, however, that LAV is not the preferred vaccine when eradication is the ultimate goal, due to uncontrolled spread of vaccine virus and incompatibility with differential detection of infected animals in the vaccinated population.

ii. Inactivated vaccines

Following the emergence of several BTV serotypes, inactivated BT vaccines became the preferred type of vaccine in Europe. Inactivated vaccines were produced at industrial scale for several but limited serotypes. The most recent European BT vaccination campaigns have exclusively used inactivated vaccines. They are very safe although mild, transitory and painless local reaction at the site of injection as well as slight fever have been sometimes reported after vaccination. If produced properly, these vaccines can be highly efficacious. Their inherent potential disadvantages include high costs of production, as vaccination requires large amounts of antigen, and, at least for many of those, the need for booster immunizations as inactivated vaccines generally induce a relatively transient immunity. Currently not many vaccine doses are available in the market, most companies producing BTV inactivated whole-virus vaccines require to place the order many months in advance so they can introduce it in the production program.

iii. Vaccination

To reduce the virus circulation, it is necessary to vaccinate the 80%-100% of susceptible animal population. Only after 5 years of vaccination of 95% of susceptible cattle and sheep, the infection may be close to the eradication levels. A vaccination campaign aiming at eradication should include extensive areas surrounding any active BT outbreak. Such campaigns should also take into account climate, geography, and the abundance of suitable insect vectors and susceptible wildlife animals. All these factors are important for vaccination outcomes and for the inter-seasonal re-emergence (persistence) of BTV infection in an area. Successful control also requires restricting movement of viraemic animals between BT-affected and BT-free zones. In the absence of vaccination, BTV can persist for several years in the region becoming endemic with low level of prevalence of infection. Most of the BT vaccination campaigns implemented in these years have been on a compulsory basis funded by European or National authorities. In recent years, the insurgence of new human and animal health emergencies and the reduced number of BTV incursions in Europe, have decreased the attention of European Authorities to BT and have induced National Authorities to support vaccination campaigns on a voluntary basis. Where implemented, vaccination on a voluntary basis hardly reached high rates of participation, actually in most cases the percentage of vaccinated herds is rather low (less than 40%). So, in case of voluntary vaccination, the disease will persist and indirect (due to movement restrictions) and direct costs (due to residue animal mortality) won't be reduced at least at National level. The vaccinated herd will indeed benefit of the vaccination. Animals can be moved and will be protected from the clinical form. They, however, have to be vaccinated every year to keep protection.

Compulsory vaccination could include all livestock species, only most susceptible species/animals or animals which have to be moved. If compulsory vaccination involves:

- **animals to be moved:** losses due to movement restrictions will be prevented but BTV will be maintained (and spread) in the environment; direct and indirect costs due to BTV infection in susceptible animals will still impact the livestock industry.

- **sheep only:** vaccinated animals will be protected from clinical signs but BTV will still circulate and its adverse impact on animal movement and trade won't be reduced.
- **all livestock animals (sheep-goats-cattle-buffaloes):** the virus circulation will be progressively reduced depending on the achieved immune status of population. Both direct and indirect costs due to BTV infection will be reduced or prevented.

iv. Assess efficacy of vaccination

Assess the efficacy of a vaccination campaign might be important to justify the cost of vaccination and identify potential weaknesses in the programme. As said before, vaccination will reduce the rate of spread of BTV if a sufficiently high coverage (>80 %) is achieved. Furthermore, the infection may be close to the eradication levels only after 5 years of vaccination of 95% of susceptible cattle and sheep. Although both humoral and cell-mediated immunities are involved, the most feasible method to assess the efficacy of vaccination is serum neutralisation when dealing with bluetongue. The neutralizing antibody response is in fact the most relevant indicator of protection. Nonetheless, not all laboratories are able and have the facilities to perform this assay. Most importantly, as no DIVA vaccines are commercially available, differentiating immunity due to vaccination from that due to infection is not possible and requires additional PCR testing for viremia to monitor the prevalence of BTV infected animals in a population vaccinated with inactivated or LAV vaccines. For these reasons and for the fact that organising and implementing a monitoring plan is time consuming, costs money and requires personnel, assessing the efficacy of vaccination is in our opinion not practical for the region. Additionally, it is known that numerous BTV vaccination campaigns implemented in the last decades using either LAVs or inactivated products have been successful in controlling and, in some circumstances, eradicating the infection.

c) Control of movements

The EU regulations establish that if BTV is confirmed, a restricted zone, including a protection zone (with a radius of at least 100km) and a surveillance zone (with a depth of at least 50km beyond the protection zone) have to be declared. The movement of susceptible animals, semen, ovum or embryos out of a restricted zone should be banned, except under exemptions (or a health certificate if to another Member State) to limit the risk of further spread. Exemption conditions take into a range of accounts factors as defined in Annex 3 of the Commission Regulation (EC) No 1266/2007 and amended by the new Animal Health Law which will be in force from next April. Movements of susceptible animals may be allowed (i) within and between surveillance and protection zones where the same serotype(s) is/are involved, (ii) if animals show no signs of disease on the day of transport, and (iii) if movements do not involve crossing a disease-free area. When BTV circulation is confirmed in a premise, the Chief Veterinary Officer has to declare a control zone with movement restrictions around the infected premises. No-one is allowed to move a susceptible animal, carcase, ovum, embryos or semen to or from premises in a control zone. Animals in the area are allowed to move only for slaughtering. Restrictions in the municipalities with active infection are withdrawn 60 days after the last case of BT. Due to the uncertainties regarding the epidemiology of BTV infection and the putative role of persistently infected (carrier) cattle in the perceived global dissemination of

BTV, the imposition of bans on the movement of susceptible livestock following the discovery of BTV circulation is deemed necessary to prevent the spread of BTV but has a significant economic impact on the agricultural industry in itself. For this reason, each country may define a specific strategy for the animal movement control according to particular objectives which range from favouring the livestock industry needs to eradicating the infection. Each decision will obviously entail different consequences on infection spread at local or National level. In any case, every movement control policy should be planned in accordance with the capacity of the surveillance system to identify the virus circulation.

CURRENT BALKAN SITUATION IN REGARDS TO BLUETONGUE

In the Balkan region, the BTV situation is still confused. According to the implemented control measures as reported in the CVO's meeting, it can be hypothesised that BTV-4 is still circulating in the region and spreading by contiguity through vectors. Restricted areas have been defined and, from there, animal movements are banned all year round. Not having in place either coordinated surveillance plans or vaccination program, BTV-4 can though spread without any barrier in the region. In these conditions, control measures can be implemented only after the appearance of clinical signs and, in case of new incursions of new strains/serotypes from Greece, no systems to early detect them and limit their spread will be in place. In addition, as no or scanty information is available on the presence and dynamic of vector population, vector free season areas can't be defined and movement restrictions have to be applied all year round without any possible seasonal exemption. In the following paragraphs an attempt to describe possible scenarios according to different control strategy adopted has been made.

POSSIBLE SCENARIOS

Scenario 1: No measure in place or animal movement restriction as the only adopted control measure

BTV-4 can spread without any barrier. It will keep circulating and spreading in the region by contiguity through vectors. Restriction zones will probably expand. In our experience, a BTV serotype is generally capable of causing outbreaks for at least 3 consecutive years. After one year of circulation, a high percentage of livestock animals is still naïf and susceptible to infection. Thus, one of the possible consequences of this scenario is that, in the coming months BTV-4 starts causing disease earlier and for longer period. It is likely that BTV-4 will infect an important percentage (30%) of susceptible animals causing disease in sheep and boosting the virus burden in the environment exacerbating the clinical signs. The risk of new BTV strain/serotype introduction from Eastern corridor remains high, and in case of new incursions BTV can spread without control causing clinical cases in livestock animals. The control measures will be adapted only after the appearance of clinical signs. Movement will be restricted from larger areas but also allowed within larger homologue areas. International trade of ruminants will be allowed only after quarantine or insecticide treatment and RT-PCR. Not having knowledge of the presence and dynamic of vector population, movement restriction will be in force all year round.

Scenario 2: Animal movement restriction and entomological surveillance

BTV-4 can spread without any barrier. It will keep circulating and spreading in the region by contiguity through vectors. Restriction zones will probably expand. In the coming months BTV-4 starts causing disease earlier and for longer period. It is likely that BTV-4 will infect an important percentage (30%) of susceptible animals causing disease in sheep and boosting the virus burden in the environment. The risk of new BTV strain/serotype introduction from Eastern corridor remains high, and in case of new incursions BTV can spread without control causing clinical cases in livestock animals. The control measure will be adapted only after the appearance of clinical signs. Movement will be restricted from larger areas and international trade of ruminants will be allowed only after quarantine or insecticide treatment and RT-PCR. ***However, abundance and dynamic of the vector population will be known; vector free season areas will be defined allowing animals to be moved during the vector free season.***

Scenario 3: Animal movement restriction, BTV-4 vaccination of sheep and entomological surveillance

Although part of the susceptible animals will be vaccinated, BTV-4 will keep circulating and spreading in the region by contiguity through vectors. ***Circulation will be mostly silent as sheep will be protected from clinical signs.*** Restriction zones will probably expand. It is likely that BTV-4 will infect a relatively high percentage (30%) of susceptible animals boosting the virus burden in the environment. ***Sheep should be vaccinated every year for at least 3 years.*** The risk of new BTV strain/serotype introduction from Eastern corridor remains high, and in case of new incursions BTV can spread without control causing clinical cases in livestock animals. The control measure will be adapted only after the appearance of clinical signs. Movement will be restricted from larger areas and ***international trade of ruminants will be allowed for vaccinated sheep*** while for other ruminant species only after quarantine or insecticide treatment and RT-PCR. Abundance and dynamic of the vector population will be known; vector free season areas will be defined allowing animals to be moved during the vector free season.

Scenario 4: Animal movement restriction, BTV-4 vaccination of sheep, entomological and serological surveillance

Although part of the susceptible animals will be vaccinated, BTV-4 will keep circulating and spreading in the region by contiguity through vectors. Circulation will be mostly silent as sheep will be protected from clinical signs. Restriction zones will probably expand. It is likely that BTV-4 will infect a relatively high percentage (30%) of susceptible animals boosting the virus burden in the environment. Sheep should be vaccinated every year for at least 3 years. Through the sentinel animals, ***BTV-4 circulation will be monitored giving information on the new infected areas and newly introduced BTV strain will be detected. Authorities have the possibility to react promptly by updating restriction areas.*** Movement will be restricted from larger areas and international trade of ruminants will be allowed for vaccinated sheep while for other ruminant species only after quarantine or insecticide treatment and RT-PCR. Abundance and dynamic of the vector population will be known; vector free season areas will be defined allowing animals to be moved during the vector free season.

Scenario 5: Animal movement restriction, BTV vaccination of livestock animals, entomological and serological surveillance

Circulation of BTV-4 will be reduced with no or few BT cases in the livestock. Animals should be vaccinated for at least 3 years. Through the sentinel animals, BTV-4 circulation will be monitored giving information on the new infected areas. In addition, newly introduced BTV strain/serotype will be detected. Authorities have the possibility to react promptly by updating restriction areas and plan a vaccination campaign against the newly introduced strain preventing the spread of the virus. ***Restriction zones will be probably reduced. National and international trade will be allowed.*** Abundance and dynamic of the vector population will be known; vector free season areas will be defined allowing animals to be moved during the vector free season.

PROPOSED COHERENT STRATEGIES

INTRODUCTION

After describing the possible scenarios and the relative potential consequences according to the implemented control measures, in the following paragraph a strategic control program for Balkan countries, which in our opinion best fits with the region exigence in term of costs and benefit, is proposed. Like most BT control strategies, the proposed plan is based on surveillance, restriction and vaccination. Bluetongue has demonstrated that the infectious agents present in Turkey, Middle East, Asia can easily adapt to the Balkan peninsula, which should be considered as a unique entity, as far as the epidemiology of animal diseases is concerned. Therefore, **any effective strategy for the prevention and control of animal disease in the Balkan region must take into account this reality and recognise the need for regional surveillance networks that include all the Balkan countries**. Considering the actual situation of the Balkan peninsula in regards to Bluetongue, the strategy should target two main points, reduce (i) the losses caused by BTV-4 circulation and (ii) those caused by possible new BTV incursions.

PROPOSED SURVEILLANCE AND VACCINATION STRATEGIES

a. Protect all sheep from clinical signs and early detect re-occurrence or new serotype introduction

To early detect re-occurrence or new serotype introduction the entire Balkan region should be divided in 45X45 km^2 grids. In each grid, 58 animals should be selected and tested monthly. The animals subject to surveillance should be unvaccinated and BTV negative. This surveillance strategy should be applied in the entire Balkan region except the risky area bordering Greece where an intensive surveillance activity should be put in place in all the municipalities in a radius of 50 km from the Greece border. In this area a reinforced surveillance program based on the monthly bleeding of 58 sentinel animals in each of 20X20 km^2 grid should be implemented. Through the monthly testing of sentinel animals, BTV circulation will be monitored within the entire Balkan region, providing information on the circulating BTV and eventual newly introduced BTV strain allowing national authorities to update the size of the restriction zones. The reinforced surveillance

will detect new BTV introduction giving the possibility to promptly react by implementing *ad hoc* control measures and reduce the risk of spreading all over the Balkan area. The vaccination of all sheep against the known circulating serotypes will protect the vaccinated animals from clinical signs cutting down the direct costs due to BT. Nonetheless, BTV will still circulate with adverse impact on animal movement and trade. Restriction zones will expand. International trade will be allowed just for vaccinated sheep, while for unvaccinated ruminants it will be allowed only after quarantine or insecticide treatment and RT-PCR**.**

b. Protect restocking sheep from clinical signs and early detect re-occurrence or new serotype introduction

To early detect re-occurrence or new serotype introduction, the entire Balkan region should be divided in 45X45 km2 grids. In each grid, 58 animals should be selected and tested monthly. The animals subject to surveillance should be unvaccinated and BTV negative. This surveillance strategy should be applied in the entire Balkan region except the risky area bordering Greece where an intensive surveillance activity should be put in place in all the municipalities in a radius of 50 km from the Greece border. In this area a reinforced surveillance program based on the monthly bleeding of 58 sentinel animals in each of 20X20 km^2 grid should be implemented. Through the monthly testing of sentinel animals, BTV circulation will be monitored within the entire Balkan region, providing information on the circulating BTV and eventual newly introduced BTV strain allowing National authorities to update the size of the restriction zones. The reinforced surveillance will detect new BTV introduction giving the possibility to promptly react by implementing *ad hoc* control measures and reduce the risk of spreading all over the Balkan area. The vaccination of the restocking sheep only against the known circulating serotypes will protect young and most valuable animals from clinical signs. Part of the direct costs will be cut down but those derived from infection in adult non immunised animals will still be possible. BTV will keep circulating with adverse impact on animal movement and trade. Restriction zones will expand. International trade will be allowed just for vaccinated sheep, while for unvaccinated ruminants it will be allowed only after quarantine or insecticide treatment and RT-PCR.

c. Protect restocking sheep and monitor BTV circulation

This strategy aims to monitor the BTV circulation. It won't be able to early detect re-occurrence or new serotype introduction. It is also based on active surveillance of 58 sentinel animals in 45x45km^2 grids. In contrast with the previous strategy, the sentinel animals should be bled four times a year. The animals subject to surveillance can be also randomly selected among animals between 8 and 12 months old, which have lived in the area under surveillance for at least six months. In alternative, the sampling can be carried out on animals selected at the slaughterhouse. In this case, sampling both blood with and without EDTA will be necessary to test by RT-PCR the samples positive to the serological test. These surveillance strategies should be applied in the entire Balkan region except the risky area bordering Greece where an intensive surveillance activity should be put in place in all the municipalities in a radius of 50 km from the Greece border. In this area a reinforced surveillance program based on the monthly bleeding of 58 sentinel animals in each of 20X20 km^2 grid should be implemented. Through the repeating testing of sentinel animals, BTV circulation will be monitored

within the entire Balkan region, providing information on the circulating BTV and allowing National authorities to update the size of the restriction zones. The reinforced surveillance will detect new BTV introduction giving the possibility to promptly react by implementing *ad hoc* control measures and reduce the risk of spreading all over the Balkan area.
The vaccination of the restocking sheep only against the known circulating serotypes will protect young and most valuable animals from clinical signs. Part of the direct costs will be cut down but those derived from infection in adult non immunised animals will still be possible. BTV will keep circulating with adverse impact on animal movement and trade. Restriction zones will expand. International trade will be allowed for vaccinated sheep only while for other ruminant species it will be allowed only after quarantine or insecticide treatment and RT-PCR.

PROPOSED MOVEMENT RESTRICTION STRATEGY

When thinking of BTV control strategy, it should be kept in mind that a strategy only based on either direct control measures (movement restrictions) or vaccination would not be capable of limiting the spread of infection effectively. A successful control in fact requires the contemporaneous appliance of both, vaccination of susceptible animals and restricting movement of viraemic animals between BT-affected and BT-free zones. Control and prevention of BTV infection in ruminants are very important due to the economic impacts of disease and animal movement/trade restrictions

As previously reported (see the paragraph on Movement control), all EU member states are required to enforce restrictions on animal movements once a BT outbreak has been confirmed. All needed actions with regards to the control, monitoring, surveillance and restrictions on movements of certain animals of susceptible species in relation to bluetongue are now stated in the Council Directive 2000/75/EC and Commission Regulation (EC) No 1266/2007 of 26 October 2007 which will be replaced by Regulation (EU) 2016/429 from 20 April 2021. In brief once BTV circulation is confirmed, there is a complete ban on movement for all farms within a radius of 20 km of the affected farm. This area is known as the Control Zone. Outside this, two zones are defined that correspond to two decreasing levels of infection risk: a Protection Zone, which extends to a radius of 100 km beyond the detected farms, and a Surveillance Zone which occupies a further 50 km outside the Protection Zone. The movement of susceptible animals, semen, ovum or embryos out of a restricted zone should be banned, except under exemptions (or a health certificate if to another Member State) to limit the risk of further spread. Exemption conditions take into a range of accounts factors as defined in Annex 3 of the Commission Regulation (EC) No 1266/2007 and amended by the new Animal Health Law which will be in force from next April.
Dealing with vector borne diseases especially when vaccination is the principal control measure, direct losses are virtually negligible but indirect losses due to movement restrictions become substantial. Moreover, losses incurred as a result of movement restrictions also have an impact on farmers in free areas who are dependent on animal movement for their livelihoods as well. In accordance with the EU rules, however, it is possible to modulate the strategy according to the farmer/livestock industry needs. In the above proposed strategies, Balkan region was considered as a unique entity. Considering the current BTV situation, all region could be regarded as BTV-4 restricted area. This will allow free ruminant movements within the area (or at least within the

national borders) and most of the costs due to National (and Balkan) trade barrier will be reduced. While this is a required condition if proposal c will be adopted, for the other two proposals restriction zones could also be defined depending on the monitoring results.

Finally, it should not be forgotten that Bluetongue is a vector-borne disease and, as such, several aspects of its epidemiology could be better understood by knowing the distribution, activity and behaviour of its vectors. The seasonal nature of BTV transmission in the Balkan countries is likely due to the seasonal activity of its *Culicoides* vectors. The current EU legislation allows Member States to declare 'vector-free period', based on **entomological surveillance**, which reduces the testing requirements for animal movement. Entomological surveillance however has its own costs. In our opinion an entomological surveillance program on the Balkan countries could be implemented only if the Region has large livestock areas in which winter temperatures are low (below 12° C on average) for periods longer than three months.

LABORATORY TESTING

Serum samples should be tested by c-Elisa as screening test. C-ELISA positive samples should be confirmed by serum neutralization test (SNT) which is able to determine the neutralizing titers and identify the circulating serotype. To assess if a sick animal is infected by BTV or if it is still viraemic and at risk to transmit the infection, EDTA blood samples should be tested by qRT-PCR. All laboratories should be able to perform C-ELISA and qRT-PCR, at least one lab/country should be able to perform SNT.

DATA AND INFORMATION SHARING

The importance of data and information sharing for the prevention and control of infectious diseases has long been recognised. In recent years, public health emergencies such as SARS- CoV- 2, avian influenza, drug-resistant malaria, and Ebola have brought renewed attention to the need for effective communication channels between health authorities, particularly in regional contexts where neighbouring countries share common health threats. In this regard BT is not an exception. Timely sharing of BT surveillance data enables better preparedness and response, nationally and regionally. A regional BT control program however to be functional needs to (i) strengthen national ability for early detection, complete recording, timely reporting, regular analysis and prompt feedback; (ii) reinforce national and supranational laboratory skill to confirm BTV cases; (iii) strengthen capacity for emergency preparedness and response at all levels.

The correct implementation of the surveillance programme is based on the knowledge of the distribution of animals and holdings within each country. Sentinel animals, as well as animals randomly selected to be tested under the surveillance programme, need to be representative of the geographical area. For this reason, a database including the identification and distribution of herds by species as well as the ownership and the updated registration of animal population in the holding, is required.

This information is even useful to plan surveillance activities to be implemented after the confirmation of virus circulation. The number of animals tested out of the number of animals under the programme is a good indicator of the activity progress. If the percentage of tested animals is

under an acceptable value (for example 80%), the status of the geographical area can be considered as epidemiological unknown and, then, treated as an infected area.
Once the surveillance programme is implemented, a quick, exhaustive and standardized flow of data on surveillance outputs should be created to guarantee the management of disease emergencies. The system should collect data on notification and follow up of disease outbreaks and record the lab results of surveillance activities. These data are useful to understand the timing of disease onset and its spread. For the lab data, the minimum dataset should include the date of sampling, the id of the animal tested and the serological and virological results. Even the data of entomological activities should be collected and made promptly available to the veterinary services.
The results of the vaccination campaign should be recorded at single animal id or at least at the herd level. For each herd, the number of vaccinated animals, animals with primary vaccination (initial + booster) should be recorded in the vaccination programme. National information systems able to support and record veterinary service surveillance and control activities as well as lab results can be supportive for data sharing between countries. A minimum set of information collected through the surveillance and control activities should be promptly shared between all the countries of the Balkan Peninsula in order to prevent and control BTV circulation. As an example, the immediate notification at regional level of all the information related to the onset of suspected outbreaks is essential to gain timely and effective control measures as well as sharing information on vaccination campaign progress and virus typing. A minimum data set to be shared should include: (i) number of suspect and confirmed outbreaks with the relative number of case by farm and municipality; (ii) number of animal vaccinated by farm and by municipality; (iii) information on virus typing.

COST BENEFIT ANALYSIS

Because of the potential severe economic consequences, that it can cause in epidemic situations, BT is regarded as “notifiable disease” by the World Organisation for Animal Health (OIE) and listed in categories C+D+E of the new European Animal Health Law. A notifiable disease is any disease that has to be reported by law to government authorities. Several estimates of the impact of Bluetongue have been reported over the years at both, global and national level. A global costing of BT has been estimated to be around 3 billion US$. This is in line with the costs (from 85 million to 1.4 billion US$,) recently assessed at national level in Europe following the BTV-8 incursions. Losses due to any livestock disease can be ordered as losses in production (direct losses), expenditure and lost revenue (indirect losses). The former may be visible losses, such a reduced milk yield or increased mortality, weight loss, reduced fertility rate, abortion, reduced meat production efficiency and death. Indirect losses include costs of vaccines or lost revenue, such as through trade restrictions limiting access to higher value markets. Knowing these processes and quantifying production losses are prerequisite to develop relevant economic models particularly when drawing control strategy and allocating resources.

A cost benefit analysis (CBA) is a process used to measure the benefits of a certain decisions relative to the associated costs and allows to compare the economic efficiency of alternatives in disease control.

The elements necessary for a comprehensive and reliable CBA shall at least involve:

- Decision on alternatives for disease control including the details on specific measures that are associated to each alternative;
- Assessment of the associated costs in monetary terms;
- Identification of benefits from the implemented measures;
- Assessment of the benefits in monetary term;
- Estimate of the impact over time that will be covered with the CBA.

It is then understandable that comprehensive and reliable CBA to disease control options requires relevant and reliable data on the expected costs and losses. Assurance of the availability of such data will require knowledge on the particular disease (initial epidemiological parameters that are currently applying in the country, measures to control the disease and their impacts, conditions under which measures will proof to be correct).

In previous paragraphs, different scenarios based on different ways of facing BTV circulation/incursions have been hypothesised. Movement restriction due to BTV circulation is definitely the most significant economic loss because of BT. Regarding the hypothesised scenarios, costs will include those for serological and entomological surveillance plans (sampling, serological and virological testing, vector identification), those for outbreak investigation and implementation of control measures and those needed for establishing vaccination campaigns. Regarding BTV infection, costs will depend on the severity of clinical forms, which may be acute, sub-acute or just mild. The related outcomes (sudden death, prolonged illness, full or partial recover of productive capacities or only asymptomatic or remise of symptoms in few days) will outline the associated costs (market value of dead animals, production losses minus reduced farm costs, costs of culling, costs of carcass disposals, production losses, mainly milk, abortions, stillbirths' lambs and reduced fertility).

The expected consequences (or results) of each scenario shall be defined based on assumptions and official field and scientific resources. In doing this, an important part will be played by BTV strain virulence, preparedness and timely response of the competent authority, vaccination coverage, awareness of farmers and their willingness to support the control strategy. In order for the CBA to provide reliable results, the assumptions, costs and the expected results thereof must be adjusted to the country or region specificities.

CONCLUSIONS

In this document a comprehensive set of information on BTV biology and epidemiology and scenarios have been provided to give National Authorities the tools for selecting the BTV control strategy which best fits their country needs. In countries where BT is endemic, the economic impact largely depends on loss of trade due to restrictions and the costs of surveillance, health testing and vaccination. Three different control strategies have also been proposed. Pros and cons of each proposal have been detailed. These proposals approached the problem considering the Balkan region as unique entity in which BTV-4 is endemic. In our opinion, this approach is far more convenient in terms of costs/benefit compared to a National strategy. Costs of surveillance and

vaccination programs have been minimized trying to ensure the efficacy of the adopted measures in compliance with the EU rules. The surveillance programs of proposals “a” and “b” have been based on early detection of BTV. A highly sensitive and constant monitoring on current and new BTV strain circulation in the region allows a prompt reaction by Authorities. In the proposal “c”, the monitoring activities is less demanding. Costs are reduced but the detection window will be longer with consequent repercussions on the BTV spread in the region. In all proposals, a timely and higher sensitive surveillance program in the Greece borders has been suggested. Most introductions of BTV into the Balkan Peninsula (and Europe) in the last decade have occurred via the Middle-East – Turkey ‘gateway’. It is therefore sensible to make the most efficient use of limited surveillance resources by targeting this known route of viral introduction into the Balkans. The proposed vaccination strategy including all or restocking sheep only, will significantly reduce direct economic losses caused by morbidity, mortality, reproductive problems, and reduced milk production. A regional approach will also guarantee an effective rapid alert system and a coordinated control strategy will avoid the spread of the virus in the region. To achieve these goals at regional level, however, data sharing becomes crucial. Although it may require a great deal of effort, resources, and collaboration, in this context data sharing is strongly encouraged. It will promote more connection and collaboration between countries, which can result in valuable common strategy and reduced monetary investment for vaccination campaign and surveillance programs. Better and more widely disseminated information can lead to informed decision making for effective and more affordable policies with great benefit for all involved actors.