

Information management and *ante-mortem* inspection procedures for the emerging diseases control: Experiences acquired in the epidemiological surveillance of bluetongue and lumpy skin disease

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Abstract

The spread of exotic, emerging and re-emerging diseases, has become, in the last years, one of the most important threats to the animal productions and public health, representing a new challenge for the European Community. In a global-market framework, where trade and contacts between countries are simplified, effective and well-developed surveillance systems are necessary. Multiple factors are, in fact, associated with the emergence of new, known or exotic diseases in this new economic panorama and for these reasons controls on animal imports, traceability and timeliness detection of infected animals should be considered the basis of a sound surveillance. In this work, we focused our attention on the management of Bluetongue and on the risk of introduction of the Lumpy Skin Disease in Italy, in order to describe the national and European surveillance systems for these diseases. In particular, we underlined the crucial role of information that reach the Official Veterinarian at the slaughterhouse concerning the epidemiological situation of the sending countries. Information that are important for the management of the *ante-mortem* inspection and for increasing the awareness of the Veterinary Inspectors of their role in the surveillance.

Introduction

Emerging and re-emerging diseases represent a new challenge for the Veterinary Public Health Services (VPHSs) (European Court of Auditors, 2016; Rodríguez Prieto *et al.*, 2015). The complexity of this theme is linked to severe difficulties for the VPHSs to properly define, control and manage all the factors involved in the spread of those diseases, which are characterized by different mechanisms of introduction, including: the movement of infected live-stock, the passive or active movement of infected vectors and other possible routes (Wilson & Mellor, 2009). We carried out an analysis on the diseases surveillance systems developed in Italy and their interactions with the European database, in order to assess the management of emerging and re-emerging diseases. We based our analysis on two diseases such as Bluetongue (BT), already circulating in Italy, and Lumpy Skin Disease (LSD), recently detected in the Balkans (Mercier *et al.*, 2017), to establish factors implicated in their spread. Our primary concern was emphasize the role of the epidemiological data and surveillance information to guide procedures at slaughterhouse and bring to the attention current practices for *ante-mortem* inspection (Vågsholm, 2014), using the two diseases above as an example.

Materials and Methods

Recent literature reviews, reports and data on emerging and re-emerging diseases surveillance, risk factors and control measures have been considered in this study. Through EFSA reports (EFSA Panel on Animal Health and Welfare, 2017; EFSA Panel on Animal Health and Welfare *et al.*, 2017; European Food Safety Authority, 2013; European Food Safety Authority, 2017), European and International database, which include: the Trade Control and Expert System (TRACES) (online at ec.europa.eu/food/animals/traces_en); the Animal Disease Notification System (ADNS) (online at ec.europa.eu/food/animals/animal-diseases/not-system_en); the Global Animal Disease Information System for Emergency and Prevention (EMPRES) (online at empres-i.fao.org/eipws3g/) and the World Animal Health Information System (WAHIS) available at www.oie.int, we collected and analysed information about BT and LSD distribution. Italian networks, such as the National Information System for the Notification of Animal Disease (*Sistema Informativo Malattie Animali Nazionale*:

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SIMAN), available at www.vetinfo.sanita.it and the National Animal Identification and Registration System (*Banca Dati Nazionale*: BDN) available at www.izs.it, have also been consulted. Moreover, in accordance with the scope of this article, colleagues working in different public health sectors have been contacted via email to understand how the problem of emerging disease is handled at an authority competent level (*e.g.* Local Health Unit).

Results

Distribution of Bluetongue

First described in 1905 in South Africa, BT actually has a very wide distribution but was historically limited between 40° to 50° north and 35° south, in subtropical and tropical climates. In the last years, there have been incursions beyond its normal geographic distribution, where different strains have moved between geographic regions and adapted to different species of vectors

between *Culicoides* genus (Wilson and Mellor, 2009). These adaptations, the discovery of new serotypes with unpredictable virulence and clinical implications made BT a disease of concern for animal productions and trade (United States Department of Agriculture, 2016). Since 2001, BT is circulating in Italy and, as reported in the national surveillance system SIMAN, from January 2016 to May 2017 there have been 1206 outbreaks registered. The majority of cases were detected in Veneto, Campania, Sicily and Basilicata, but overall 17 regions were involved (Table 1). Actually, BTV-1 and BTV-4 are the serotypes more present in the area and although attempts have been made to identify the route(s) of introduction of these viruses by tracing animal imports, analysing sequence data and modelling meteorological conditions, their mechanism of introduction remain unclear. Moreover, the existence of a novel BTV serotype circulating in healthy goats in Sardinia was recently described, but no information about its origin have been reported yet. Sera from the infected goats failed to neutralize reference strains of the classical BTV

Table 1. Detailed bluetongue circulation in Italy from January 2016 to May 2017.

Region	Serotype	N. cases
Abruzzo	BTV-4	24
	BTV-1	1
Basilicata	BTV-4	98
	BTV-1	17
	unknown	2
Calabria	BTV-4	48
	BTV-1	5
Campania	BTV-4	127
	BTV-1	36
Emilia Romagna	BTV-4	27
Friuli Venezia Giulia	BTV-4	32
Lazio	BTV-4	39
	BTV-1	9
Lombardia	BTV-4	5
Marche	BTV-4	9
Molise	BTV-4	62
	BTV-1	1
Apulia	BTV-4	44
	BTV-1	14
Sardinia	BTV-1	26
	BTV-4	14
Sicily	BTV-4	107
	BTV-1	20
Tuscany	BTV-4	38
	BTV-1	1
Trentino A.A.	BTV-4	6
Umbria	BTV-4	9
	BTV-1	3
Veneto	BTV-4	396
		1206

serotypes and of the recent BTV-26 and BTV-27. In addition, the gene segments of this new serotype (BTV-X ITL2015) have not been detected previously, but more in-depth investigation is needed (Savini *et al.*, 2017). It is assumed that animal movement, *Culicoides* active and passive flight are the main patterns of the BTV spread.

Distribution of lumpy skin disease

Another emerging disease of increasing interest for the European surveillance is the LSD. Originally confined to sub-Saharan Africa, it arrived in the Middle East in 2000 and reached Turkey in 2013, showing the potential of further spread (Coetzer and Tuppurainen, 2004). The Lumpy Skin Disease Virus (LSDV) is highly host specific and causes diseases only in cattle (*Bos indicus* and *Bos taurus*) and water buffalo (*Bubalus bubalis*). It had spread quickly between border territory of Turkey and Greece, affecting a wide range of cattle: until 31 December 2016, 4,676 animals have been infected in Turkey; 4,371 in the Former Yugoslav Republic of Macedonia (FYROM); 4,315 in Albania; 2,019 in Kosovo and 994 in Greece (Figure 1). In July 2016, considering the rapid distribution of the disease in the Balkans, the Italian Ministry of Health (MOH) transmitted a LSD-Warning (Ministero della Salute, 2016a) with informative elements on the disease, emphasizing the importance of a rapid notification and declaring increased control measures on traded animals from border regions of affected countries. Subsequently, a MHO Notation for prevention of virus introduction in the national territory, described sanitary measures to adopt (Ministero della Salute, 2016b).

Discussion

It is important to underline that imports of live animals from countries affected by LSD are very low, due to movement limitations; on the contrary trade with at-risk countries is still present. From 2014 to 2016, animals imported for being slaughtered were 2290 from Hungary, 701 from Romania, 467 from Croatia, 35 from Lithuania and a lower amount from Latvia, Estonia and Greece.

About BTV-zones, instead, a large number of cattle imported for slaughter came from France, Slovenia and Spain and all these territories are affected by different serotype of BTV (BTV 1-2-4-8-16 in France; BTV-4 in Spain and Slovenia). Considering that *Culicoides* and traded animals are the main cause of the BT spread, it is crucial for the surveillance to cross etiopathological and epidemiological data, also at a slaughterhouse level.

Clinical signs and laboratory confirmation of BT

Clinical signs of BT in cattle are usually limited to fever, increased respiratory rate, lacrimation, salivation, stiffness, oral vesicles and ulcers, hyperesthesia, and a vesicular and ulcerative dermatitis. Highly characteristic lesions are usually obvious in severe clinical infections but may be barely visible in mild or convalescent cases, therefore Veterinary Inspectors should be aware of the risk of neglecting suspect cases. Between all the laboratory tests that can be performed, the real-time polymerase chain reaction (PCR) techniques allow for more rapid and sensitive testing. Reverse-trans-



Figure 1. Lumpy Skin Disease outbreaks notified in Europe and number of animals affected in 2014-2016.

scription (RT-PCR) technology has permitted rapid amplification of BTV cDNA in clinical samples, and RT-PCR-based procedures are now available. These procedures augment the classical virological techniques to provide information on virus serogroup, serotype and topotype. About serological tests, responses appear some 7-14 days after BTV infection and are generally long-lasting. A monoclonal antibody-based competitive enzyme-linked immunosorbent assay to specifically detect anti-BTV (serogroup) antibodies is recommended. Procedures to identify and quantify the BTV serotype antibodies are more complex, being typically based on neutralisation tests (OIE, 2017).

Clinical signs and laboratory confirmation of LSD

About LSD, it is described as an acute or unapparent cattle disease where, at an initial stage, infected animals develop a biphasic febrile response (2-4 weeks after exposure to the virus) associated with salivation, lacrimation and nasal discharge. In the majority of cases, the superficial lymph nodes become enlarged and, after the onset of fever, skin nodules appear, covering the animals. Oedema, lameness and ulcerative lesions in the respiratory tract may also occur. For LSD, the mode of transmission has not been established fully, but has been demonstrated by *Aedes aegypti* mosquito and certain types of ticks, moreover, infected saliva and milk may contribute to the spread of the disease (Coetzer and Tuppurainen, 2004). Acute and severe cases present quite characteristic signs, but mild cases can easily be confused. In the event of suspect, the Veterinary Inspectors should consider LSD between possible differential diagnosis with other diseases, such as pseudo-lumpy skin disease (BHV-2), hypodermosis, insect bites, besnoitiosis, dermatophytosis, demodicosis, bovine dermatophilosis and onchocerciasis and take samples for laboratory confirmation. The diagnosis can be confirmed by different assays: i) the PCR is the least expensive and quickest method for detection of LSDV, where skin nodules, scabs, saliva, nasal secretions and blood are suitable samples for PCR detection of LSDV; ii) Virus isolation followed by PCR to confirm the virus identity takes longer and is more expensive but has the advantage of demonstrating the presence of live virus in the sample; iii) Electron microscopy can be used to identify the classic poxvirus virion but cannot differentiate to genus or species level; iv) Serological tests can be used, but they do not distinguish the three viruses in the Capripoxvirus genus (Sheeppox virus, Goatpox virus and LSD). The Virus

Neutralisation Test (VNT) is the only validated serological test available. The agar gel immunodiffusion test and indirect immunofluorescent antibody test are less specific than the VNT due to cross-reactions with antibody to other poxviruses. Western blotting using the reaction between the P32 antigen of LSDV with test sera is both sensitive and specific, but is difficult and expensive to carry out. Some antibody-detecting enzyme-linked immunosorbent assays (ELISAs) have been described, but none is sufficiently validated to be recommended for use (OIE, 2017). As control measures, increasing surveillance and vaccination campaigns are in place in affected country and border regions to arrest the LSD circulation.

In the light of the above, we wanted to suggest that *ante-mortem* inspection can be used to survey and identify notifiable animal diseases like BT, LSD and others. An early diagnosis, in fact, might prevent an outbreak from turning into an epidemic and inspection data might be used when documenting official freedom from notifiable diseases. Also regarding international trade, animal inspection plays a role with respect in the surveillance process. During the *ante-mortem* inspection, the correct identification of animals, transportation documents (e.g. Model IV) and food chain information (FCI) are checked (European Commission, 2004). An important document is Model IV, which is a declaration of provenance containing details about the origin and health status of animals. It consists of five parts (identification of animals, declarations for the slaughterhouse, destination site, transporter information, veterinary health certificate) and accompanies the animals throughout the period of transport. Since September

2017, it can be submitted only electronically, facilitating a rapid consultation and data storage. FCI sometimes are included or they can be in a separate form. In the FCI, according to Regulation 853/2004/EC, is specified what follow: i) status of provenance or regional health status; ii) information on veterinary treatments and diseases; iii) production performance, mortality and welfare data; iv) results of laboratory testing and v) reports about previous *ante* and *post-mortem* inspections of animals from the same holding (Alban *et al.*, 2011). All this information can be relevant for the surveillance and guide the Veterinary Inspector during the monitoring process at slaughterhouse.

The spread of emerging and re-emerging diseases highlights the crucial role of traceability both of animals and animal diseases to define useful targets for the surveillance on a risk-based approach and, on the other hand, reveals the importance of a sound veterinary service framework (Bisdorff *et al.*, 2016; Natale *et al.*, 2009). At an European level, TRACES manages all sanitary requirements on intra-EU trade and importation, enabling information exchange on traded animals and updating the food chain data. TRACES cooperates also with the Animal Disease Notification System (ADNS), which is a crucial tool to assess the current outbreaks situation in the European territory. Furthermore, ADNS shares information with the World Animal Health Information System (WAHIS) to facilitate outbreaks notification and follow up (Figure 2). Therefore, what we know on emerging disease depends on the effectiveness of these surveillance systems and, earlier, on the VPHS management at a national level.

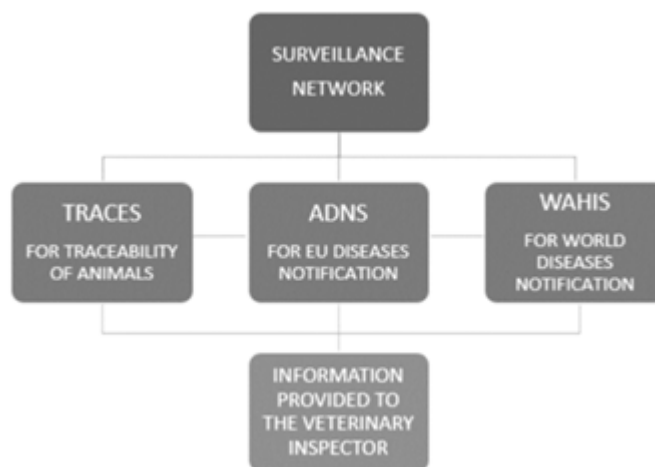


Figure 2. Network of International and European surveillance systems.

In Italy is active a contingency plan for BTV through which all the Italian competent authorities manage the surveillance activities and control measures. The national plan for BTV is handled by the Directorate General for Animal Health and Veterinary Medicinal Products (*Direzione Generale della Sanità Animale e dei Farmaci Veterinari*: DGSAF) and provides for constantly updated guidelines on BTV-control. Since 2002, a robust and organized network of sentinel animals has been established in Italy to monitor BTV circulation. During the period of vector activity, animals are tested at least once a month and positive samples are sent to the Zooprofylactic Institute *Giuseppe Caporale* of Teramo, which is the national reference centre for exotic disease, and tested by VNT/serotype specific RT-PCR to define the circulating serotype involved. Entomological surveillance is also an important part of the contingency plan, more than 250 black light traps are placed and used to verify the abundance of *Culicoides*. In the transposition of EU regulations, the definition of a protection (100 km) and a surveillance zone (150 km) around an infected area (20 km radius), was also included in the BTV control measures. The European decision 2000/75/EC considers also the possibility to define new surveillance and protection zones, evaluating the epidemiological situation of each region or country involved (Council of the European Union, 2000). Where a well-developed serological and entomological system is in place, in fact, surveillance and protection zones can be reduced in a single restriction zone with a less radius. The legislation provides also that if originating in a restriction zone for BTV, the animals are banned from leaving the zone, unless accompanied by a veterinary health certificate which confirms the animal moves under one of the agreements in Annex III of Directive 1266/2007/EC (Department for Environment Food & Rural Affairs, 2016) (European Commission, 2007). These regulations have mitigated provisions on movement restrictions, avoiding the livestock sector collapse, but Veterinary Inspectors must be constantly aware of the possible incoming risk and set out appropriate investigations.

Conclusions

Surveillance on emerging diseases represent a field of increasing interest for the VPHSs. Lots of surveillance systems are currently active for this scope and require a valid collaboration from all actors involved in the animal health and food safety chain.

The role of the Veterinary Inspector in this background is to control and examine each consignment of animals considering the epidemiological situation of the sending country or, in case of national movement, of the origin region. Different and integrated database allow Official Veterinarian to do so and to set out appropriate interventions before slaughtering. The *ante-mortem* inspection is, in fact, a crucial phase of the monitoring process and permits, on clinical signs evaluation, to outline a differential diagnosis that can be subsequently confirmed by laboratory testing. A more detailed examination is extremely recommended in case of suspect of an emerging and re-emerging disease, considering their economic impact and veterinary public health implications. For these reasons, Veterinary Inspectors should be considered in the surveillance process as an added value and properly motivated in their actions, since in the fight against emerging disease every piece of the chain is important.

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