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TITLE

A Scoring Model For Risk Characterisation And Setting Priorities In Veterinary Public Health

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Index

1 Introduction

European Union (EU) requests standards for veterinary public health and food safety in both operative procedures and official controls as stated in “White Paper on Food Safety” (COM, 1999, 719) and in regulation EC No 882/2004. These standards define the mandatory requirement and objectives for Nations within the EU. Furthermore, Standard Operating Procedures (SOPs) for Competent Authorities should be adopted in order to encounter EU requisites. To satisfy these specific aspects on food safety and veterinary public health, Lombardy regional government and the Department of Veterinary Science and Public Health (DIVET) of University of Milan initiated a shared initiative to develop a three-year program aiming to improve efficiency, efficacy and quality of the regional veterinary services. One of the tasks of this project was the development of a scoring model for risk-characterization and prioritisation concerning relevant diseases for human and animal health. This model had to take into account not only the specific characteristics of the diseases but also the geographic background of application (Lombardy region). Moreover, the scoring model had to be based on an accurate definition of the hazards and the related risks in order to provide a reliable risk-based tool for prioritisation in public health.

1.1 Definitions

In 1995, The World Health Organization (WHO) and the Food and Agriculture Organization of the United Nations (FAO) developed a manual on risk-based approaches for the management of public health hazards in food (1). In this manual and its further updates (2), FAO and WHO, through the Codex Alimentarius Commission (CAC), dictated the guidelines for risk analysis and the related definitions.

Figure 1 shows the framework of risk analysis and how risk assessment, risk management and risk communication should work together, as depicted in (1, 3).

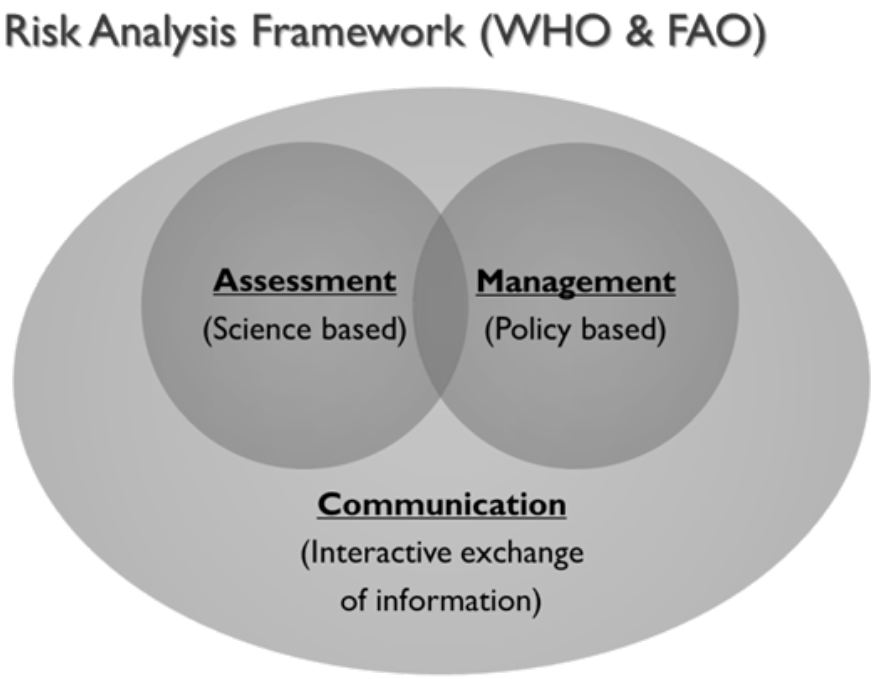

Figure 1 – Risk analysis framework (1, 3).

HAZARD: a biological, chemical, or physical agent that may have an adverse health effect (1).

RISK: a function of the probability of an adverse effect and the magnitude of that effect, consequential to a hazard (1).

RISK ANALYSIS: a process consisting of three components: risk assessment, risk management and risk communication (see Figure 1) (1).

RISK ASSESSMENT: the scientific evaluation of known or potential adverse health effects resulting from human exposure to hazards. The process consists of the following steps: *hazard identification*, *hazard characterization*, *exposure assessment*, and *risk characterization*. The definition includes quantitative risk assessment, which emphasizes reliance on numerical expressions of risk, and also qualitative expressions of risk, as well as an indication of the attendant uncertainties (1, 3-5).

- **Hazard identification:** the identification of known or potential health effects associated with a particular agent (1).

- **Hazard characterisation:** the qualitative and/or quantitative evaluation of the nature of the adverse effects associated with biological, chemical, and physical agents which may be present in food. For chemical agents, a dose-response assessment should be performed. For biological or physical agents, a dose-response assessment should be performed if the data is obtainable (1). This definition can also be adopted in veterinary public health for hazard characterization of infectious diseases.
- **Exposure assessment:** the qualitative and/or quantitative evaluation of the degree of intake likely to occur (1).
- **Risk characterisation:** integration of hazard identification, hazard characterization and exposure assessment into an estimation of the adverse effects likely to occur in a given population, including attendant uncertainties (1).

RISK MANAGEMENT: the process of weighing policy alternatives to accept, minimize or reduce assessed risks and to select and implement appropriate options. The risk management follows four step (1, 3-6):

- **Preliminary risk management activities:** preliminary risk profile and gathering of known information.
- **Evaluation of risk management options:** analysis of available system of control; resources and cost-benefit investigation.
- **Implementation of the risk management decision:** laws, regulation, controls and on-going verification.
- **Monitoring and review:** process that involves continue gathering and analysing of data and verifies control plans and the progress in risk management.

RISK COMMUNICATION: An interactive process of exchange of information and opinion on risk among risk assessors, risk managers, and other interested parties (5, 7).

1.2 Geographical Background: Lombardy region

Lombardy is an Italian region situated in the north of the nation and is bordered by Switzerland (north), Emilia-Romagna region (south), Piedmont region (west), Trentino-Alto Adige region (east), and Veneto region (east). Lombardy region, with its 9,990,604 inhabitants (8), has the largest population among Italy regions.

Lombardy is the major contributor to Italian Gross Domestic Product (GDP) in terms of agro-food production. Indeed, as reported by Regional Government Statistics, agronomic industry has a value of over 6.35 billion euros, and 70,916 farms are present within the region (9). Numerically, the most represented livestock are poultry (over 27 millions), swine (5.16 millions) and bovine (1.5 millions). However, when the share of regional contribution to the national output is considered, swine meat represents 40% of the total, milk production 37%, bovine meat 26%, poultry meat 19% and egg production 17.7% (10). Moreover, Lombardy exports, related to livestock industry, represent almost a third of all Italian exportations in the sector. Specifically, about 70% of these trades are with EU countries (value of 1,572 million of euros) and the remaining 30% with countries outside EU (value of 723 million of euros) (11).

Food industry is well-developed too, with an output value of 5.2 billion (9). In Lombardy there are over 63,000 plants for production, transformation, or trade of foods of animal origin and 2,739 of these plants are certificated at EU-level. These latters establishment employ over 28,000 people (11). Furthermore, the average number of employee for plants that work only at national level is 6.9 while plants certificated at EU-level have 49.4 employee on average (11).

These data emphasize, if needed, the strategic value of agronomic and food industries and, therefore, the importance of zoonosis control and food safety in the region.

Table 1 summarises the official status of animal diseases, in Lombardy, reported by Regional Government Statistics (9, 11).

Bovine

Disease	Official status	Frequency of the disease
Bovine tuberculosis	Free.	Sporadic (5 outbreaks in 2012, 4 in 2013).
Bovine brucellosis	Free.	Sporadic to none (less than 1 outbreak per year).
Enzootic bovine leucosis	Free.	None.
Blue tongue	Free.	None.
Paratuberculosis	Not free (novel official control plan, *DDGN N°6845 del 18/07/2013*).	About 70% of prevalence at farm-level.
Bovine spongiform encephalopathy (BSE)	Free.	None (last positive case in 2011, a 14-years old cow).
Infectious bovine rhinotracheitis (IBR)	Not free (1,699 farms with official free status).	36.50% of prevalence at farm-level (serological surveillance).
Mastitis (*S. agalactiae*)	Not free (about 4,000 farms considered free).	12 – 15% of prevalence at farm-level (spot-check surveillance).

Small ruminants

Disease	Official status	Frequency of the disease
Brucellosis (small rum.)	Free.	None.
Blue tongue	Free.	None.
Transmissible spongiform encephalopathy (small rum. TSE)	Not free.	Sporadic (17 outbreaks from 2003 to 2013).
Contagious agalactia	Not free.	Sporadic (15 outbreaks from 2009 to 2012).

Swine

Disease	Official status	Frequency of the disease
Aujeszky's disease	Not free.	21 – 27% of prevalence at farm-level.
Classic swine fever	Free.	None.
Swine vesicular disease	Free.	None.

Poultry

Disease	Official status	Frequency of the disease
Avian influenza	Not free.	Sporadic (7 outbreaks of H5N2 in 2012, 1 of H5N3 in 2013).
Salmonellosis (*S.* Enteritidis, *S.* Thyphimurium)	Not free.	*Chickens*: 5.92% (2012) – 1.42% (2013) at animal-level. *Parents*: 1.71% (2011) – 0% (2012/13). *Broilers*: 2.7% (2011) – 0.61% (2013). *Turkeys*: 0% (2011) – 1.03% (2013)

Others

Disease	Official status	Frequency of the disease
Rabies	Free.	None.
West Nile fever	Not free.	Spot-check surveillance, 2013: *Insects*: 152 controls, 7 positives. *Horses*: 440 controls, 5 clinical cases and 6 seropositives. *Synanthropic birds*: 756 controls, 2 positives (crows).

Table 1 – Regional Government Statistics on animal diseases.

1.3 Setting priorities in public health

Several Countries allocate a large amount of human and financial resources to public health. However, these assets are not unlimited, especially in case of veterinary public health, therefore, setting priorities for rational allocation of available resources is pivotal. In addition, health risks are subject to relevant changes over time, and thus, primary targets and surveillance programs should be reviewed periodically (12).

Various scoring model for prioritisation were developed during the last decades and they were based on different methods. In the specific field of public health, three approaches shows particular usefulness: multiple-criteria decision analysis (MCDA) (13-16), consensus processes (12, 17, 18), and expert frameworks (17, 19-21). Among these approaches, consensus processes can be effective methods when scientific data is absent, insufficient or contradictory (12); in public health and healthcare the most frequently applied processes is the Delphi protocol.

The Delphi protocol is a consensus process extensively used in very different fields. This protocol was developed initially for business, industry management, and government. Nevertheless, the Delphi technique is also applicable in other fields such as healthcare science (22, 23), veterinary science (24, 25), and public health (26). Furthermore, a recent method, named Formalized Consensus Process (FCP), was developed specifically for healthcare and public health (27).

1.3.1 Delphi Protocol

The Delphi protocol is a structured process developed to provide a systematic method for reaching consensus among a group of experts. This protocol can be suitable for the following major objectives (28):

- To assess information which may lead to differing judgements;
- To collect information which may produce a consensus of the group;
- To integrate judgements on issues that encompass a wide range of disciplines;
- To instruct the group about different and interrelated aspects of the problems.

The Delphi method involves several progressive steps that can be summarised as (22):

1. **Identification of the problem.**
2. **Selection of the expert panel:** during this phase is selected a group of several individuals with specific expertise about the problem under analysis.
3. **First round:** during this step opinions of the experts, regarding the issue under assessment, are collected. These statements are gathered in a specific questionnaire and, finally, the questionnaire is submitted to each participants.
4. **Second round:** experts score their degree of agreement with every statement in the questionnaire, these ranks are summarised and integrated with the questionnaire.
5. **Third round (repeatable):** experts re-score their agreement with every statement in the questionnaire, during this phase is possible to review the scoring in light of the second round results.
6. **Analysis of results:** round results are analysed to establish the degree of consensus, if the consensus is not reached the third round must be repeated until agreement among experts is fulfilled.
7. **Final report:** a document that gather the results of the process (finalised statements, guidelines, prioritisation models, etc.) and accurately recap the previous steps is produced.

A facilitator supervises the entire process, this individual provides assistance to the experts (collection of answers, feedbacks, statements, etc.) and manages the various steps of the protocol.

One of the "core" feature of the Delphi protocol is anonymously, indeed, responses and commentary during rounds are strictly anonymous in order to avoid domination of the process by one or few participants (29). Additionally, criteria that defines consensus among the experts should be clearly identified and reported before the beginning of the process itself (30).

1.3.2 Formalized Consensus Process

Formalized Consensus Process is a method of consensus developed by the Haute Autorité de Santé (HAS), a French Independent Administrative Authority (Autorités Administratives Indépendante – AAI). HAS published the initial FCP guidelines during the 2006 (31) and an update in 2010 (27).

The main objectives of FCP are to formalize agreement between experts and to identify and select the best practices. Using the FCP can be considered if at least two of the following conditions are met (27):

- Insufficient literature sources regarding specific questions or problems;
- An independent expert panel witch need to select appropriate solutions of different issues from several alternatives;
- Topics or clinical conditions easily identifiable.

Development of FCP follows five phases such as (27):

1. **Systematic review and synthesis of literature:** this step starts with the identification of scientific topics and keywords to comprise in the search. Moreover, criteria for the inclusion/exclusion of scientific papers (e.g. English-written and peer-reviewed) must be defined *a priori*. Finally, a critique analysis of search results must be performed in order to establish the degree of pertinence of the papers.
2. **Assessment of issues:** a group of experts provides a selection of issues and proposal which will be analysed. Specifically, this phase comprises two round of analysis; an intermediate feedback meeting has also to be performed. During the assessment of issues, points of agreement, disagreement or indecision are discussed too.
3. **Initial proposals:** the purpose of this step is to write the first draft of recommendations and proposals that the expert panel will further discuss and review.
4. **Discussion and improvement of proposals:** during this phase the original version of recommendations and proposals are reviewed, in particular their acceptability, applicability and readability. Furthermore, all the quotes and comments of the group of experts are collected and used to improve the initial draft.
5. **Finalisation:** this phase produces a series of documents which encompasses all the recommendation, scientific argument and final models shaped during the process.

A facilitator (as an individual or a group) follows the entire process and provide a role comparable to the facilitator of the Delphi methods.

1.4 Scoring systems for veterinary public health

Despite the availability of consolidated methods, such as consensus processes and MCDA, scoring systems for prioritisation and risk characterisation in veterinary public health are still poorly available. Moreover, available systems are usually based on expert frameworks.

During the last 15 years, different tools for prioritisation in animal health were developed. These systems had different focus, depending on their objectives, and they are reviewed in (17). Among these models, three scoring systems were settled to cover more than one distinct sector in veterinary public health: a scoring system for emerging zoonosis developed in the Netherlands (32), a scoring model by a group of EU Chief Veterinary Officers (33), and the DISCONTOOLS scoring model (34).

In 2006, the Netherlands National Institute for Public Health and the Environment developed the Dutch Emerging Zoonosis Information and Priority systems (EZIPs). EZIPs provided a scoring model for zoonosis that ranked seven categories of interest (32):

1. Probability of introduction into the Netherlands
2. Transmission in animal reservoirs
3. Economic damage, animal reservoirs
4. Animal-human transmission
5. Human-human transmission
6. Morbidity
7. Mortality

Not all the seven categories had the same importance and a coefficient of weight was applied, indeed, animal-human transmission (coefficient 0.63) and mortality (0.64) were identified as the two most important categories. Through EZIPs model 86 zoonosis were assessed (data available at http://ezips.rivm.nl/pathogens/).

In 2008, a working group of EU Chief Veterinary Officers proposed a complex scoring model for infectious diseases. This model assessed implication of animal diseases on public health, farming economy, society, and trade. The working group identified six areas of interest and four to ten criteria for each area, specifically (33):

1. Epidemiology (10 criteria).
2. Control measures (8 criteria).
3. Impact on public health (4 criteria).
4. Impact on economy (4 criteria).
5. Impact on society (4 criteria).
6. Impact on trade (4 criteria).

Criteria may be scored from 1 (best case) to 5 (worst) and each criterion has a coefficient so that the total of weighted criteria in each areas is 10 (areas may score between 10 and 50) (33).
DISCONTOOLS is an on-going project founded by EU members states that involves industry, researchers and EU public health officers. The aims of this project are:

1. To develop a disease prioritization system in order to prioritize researches in diagnostics, vaccines, and pharmaceuticals. Thus, to improve surveillance and control of animal diseases.
2. To identify gaps among the diseases assessed and to identify where further researches are needed.
3. To increase efficiency of new technologies for animal health.

In 2011, DISCONTOOLS project (DP) developed a scoring system (see Table 2) for prioritisation and gaps identification in infectious animal diseases (34, 35). This model covers six areas of interest such as disease knowledge, impact on animal welfare, impact on public health, impact on wider society, impact on trade, and control tools. Areas were subdivided by tree to ten criteria and each criteria could be ranked from 0 (best) to 4 (worst), with the exception of "control tools" where the scoring range were from +2 to -2.

Criteria	Scores					Coef	Total (score*coef)
Disease knowledge	**0**	**1**	**2**	**3**	**4**		**/100**
1. Speed of spread						2.5	
2. Score for number of species involved						2.5	
3. Persistence of infectious agent In the environment						2.5	
4. Risk of spread to susceptible populations						2.5	
5. Potential for silent spread						2.5	
6. Wildlife reservoir and potential spread						2.5	
7.Vector reservoir and potential spread						2.5	
8. Variability of the agent						2.5	
9. Understanding of fundamental immunology						2.5	
10. Host pathogen interaction						2.5	
Impact on animal health and welfare	**0**	**1**	**2**	**3**	**4**		**/100**
1. Disease impact on production						8.33	
2. Duration of animal welfare impact						8.33	
3. Proportion of animals affected suffering pain/injury/distress as a result of the disease						8.33	
Impact on public health – human health	**0**	**1**	**2**	**3**	**4**		**/100**
1. Impact of occurrence on human Health						4.16	
2. Likelihood of occurrence						4.16	
3. Impact of occurrence on Food Safety						4.16	
4. Transmissibility (spread from animals to humans)						4.16	
5. Spread in humans						4.16	
6. Bioterrorism potential						4.16	
Impact on wider society	**0**	**1**	**2**	**3**	**4**		**/100**
1. Economic direct impact including cumulative cost (e.g. Enzootic vs. epizootic)						8.33	
2. Economic indirect impact (social, market)						8.33	
3. Agriterrorism potential						8.33	
Impact on trade	**0**	**1**	**2**	**3**	**4**		**/100**
1. Impact on international Trade due to existing regulations						6.25	
2. Impact on EC Trade due to existing regulations						6.25	
3. Potential for regionalisation						6.25	
4. Impact on Security of Food supply						6.25	
Control Tools	**2**	**1**	**0**	**-1**	**-2**		**/100**
1. Appropriate diagnostics						16.66	
2. Appropriate vaccines						16.66	
3. Appropriate pharmaceuticals						16.66	
Total score							

Table 2 – DISCONTOOLS scoring model for prioritisation.

DISCONTOOLS working groups identified and assessed 52 animal diseases belonging to three categories (36): epizootic diseases (e.g. foot and mouth disease, bluetongue), food animal producing complexes (e.g. BVD, *Staphylococcus aureus* mastitis), and zoonotic diseases (e.g. bovine tuberculosis, cysticercosis).

An online database (http://www.discontools.eu/Diseases) contains all the information about scoring criteria, gap analysis, risk, and detailed characteristics of the 52 diseases analysed. The database is regularly updated on a 3-year cycle, however, novel information regarding specific diseases can be added anytime. In addition, new diseases may be included in future (37).

2 Aims of the study

The main purpose of this study was to develop a trustworthy tool for characterize risks and identify priorities of animal diseases in very specific areas (health, economic, international trading, control programs, etc.) applicable at a regional-level.

In order to identify priorities and risks of a given disease, a reliable model should consider the level of risk of that disease in all sectors of interest. Furthermore, it should facilitate the decision-making process due to a rational, scientific and clear procedure that permits quick and efficient responses when known or emerging diseases are considered. Finally, it should highlight possible gaps of information (e.g. epidemiological data, costs of control plans in the region) concerning the diseases under assessment.

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3 Materials and Methods

To define risks and priorities within the diseases of interest in Lombardy region, a scorecard was developed (see Table 4) based on the model settled by DP (34, 35).

The scoring model developed by DP evaluates a disease in the entire EU and it provides a scoring system regarding disease knowledge, impact on animal health and welfare, public health, wider society, trade and disease control tools. In contrast to DP model, the scorecard developed in this study is focused mainly on human health, food safety and economic impact of the diseases. Moreover, the target area, Lombardy region, is relatively narrow if compared to EU.

3.1 Model development

An expert panel (EP) was organized (academics and public health officers) in order to develop the scorecard and to establish a proper scoring system using DP model as core starting point. Furthermore, a guide (see Table 6 to Table 11) and a form were developed by EP to assign appropriate scores for each category (three examples of fulfilled forms are reported in the appendixes of this thesis).

The form encompasses all the information needed to assign a proper scoring, and the guide explains how to translate these information in a score from one to five.

The working group selected, among the available methods, the FCP (27) to develop the final version of the scorecard, the guide, and the form. Formalized Consensus Process is a five-step procedure which includes a systematic review of literature, an assessment of issues, initial proposals, a discussion and improvement of proposals and, finally, a validation of operational recommendations. In this specific case, the process involved the following steps:

1. Systematic review of literature concerning models for risk characterization, prioritization and management of veterinary public health. In addition, EP identified the hazards to assess as contagious diseases and a preliminary list of these diseases was selected.
2. Identification of main technical issues such as sources of information, EP meetings organisation, and lack of pre-existing models. Moreover, major areas of interest for veterinary public health at regional level were addressed, and the final list of diseases was established (see Table 3).
3. Development of a first draft of scorecard, related guide and form: comments and suggestions were collected in joint meetings between EP and several veterinary officers of Lombardy region to refine the questions requested for assessment of a single criteria (e.g. “presence of the disease”).

4. Improvement of the draft: EP further discussed with veterinary officers of Lombardy region (VOL) and established a different "weight" for the areas of interest (see Table 5), and all categories within the areas were established (see Table 4).
5. Test of pilot scorecards addressing three well-known diseases (bovine tuberculosis, bovine brucellosis, and enzootic bovine leucosis); the results were examined in a joint meeting between EP and VOL. This assembly produced the final structure of scorecard, guide, and form. The procedure for the assessment of the diseases (filling of the forms and scoring process) was also established.

3.2 Expert panel

The expert panel comprised veterinaries with either an academic or a public health management background. In detail, DIVET provided seven academics with at least 10 years of experience in research and control of infectious and/or parasitic diseases while VOL provided five veterinary officers with at least 10 years of experience in veterinary public health. Furthermore, DIVET and VOL selected one facilitator (the author of this thesis) and two collaborators with a degree in veterinary medicine and at least one year of experience in control of infectious or parasitic disease. These collaborators, alongside with the facilitator, expedited communication between VOL and DIVET outside the plenary meetings, managed the references (order and style), and provided basic assistance throughout all FCP steps and during the filling of diseases forms.
Consensus was considered to be reached when at least 9 out 12 expert (75%) agreed on a given issue.

3.3 Systematic review

Systematic review started with a simple free-form question, exactly, *are scoring model for risk characterization in veterinary public health available?*
In February 2011 an electronic research of Web Of Science (WOS) database was performed, general settings were "All years" and "All databases" while the keywords were the following: (veterinary) AND ("public health") AND ("risk characterization" OR scoring OR prioritization). As control, the same search was carried within PubMed database.
One hundred and one documents were founded in WOS (19 in PubMed); papers were considered admissible only if written in English and peer-reviewed (with the exception of documents by OIE, EFSA, and CDC), therefore, 16 papers out of 101 were excluded.

Quality assessment was conducted on the 85 articles left, a ranking system was adopted to identify pertinence of the papers, precisely:

- **Score = 0** (*no pertinence*): scientific documents completely out of topic, such as risk management in clinical veterinary medicine; epidemiology of antimicrobial resistances; new diagnostic techniques; risk factor for single aspects of a disease or for a specific problem of health/welfare of a single species.
- **Score = 1** (*low pertinence*): scoring systems regarding a specific aspect of a single given disease; general methodology and scientific opinion about risk characterization or disease prioritization.
- **Score = 2** (*medium pertinence*): scoring systems, applied in distinct sectors (e.g. human health, economics and animal welfare) for a single diseases or for more than one disease but in a single sector; specific scientific opinion concerning prioritization in zoonosis or food safety.
- **Score = 3** (*high pertinence*): single scoring models able to provide risk characterization in very distinct sectors and appropriate for diverse veterinary diseases of interest in public health.

67 documents scored 0, 15 scored 1, 3 scored 2 (38-40) but no papers reached score 3; the results of the systematic review are collected in the Appendix D and the flowchart is illustrated in Figure 2. Since none of the scientific works founded were classifiable as high pertinent, EP adopted the DP model as starting point and the methodologies reviewed in (17) as source of additional information.

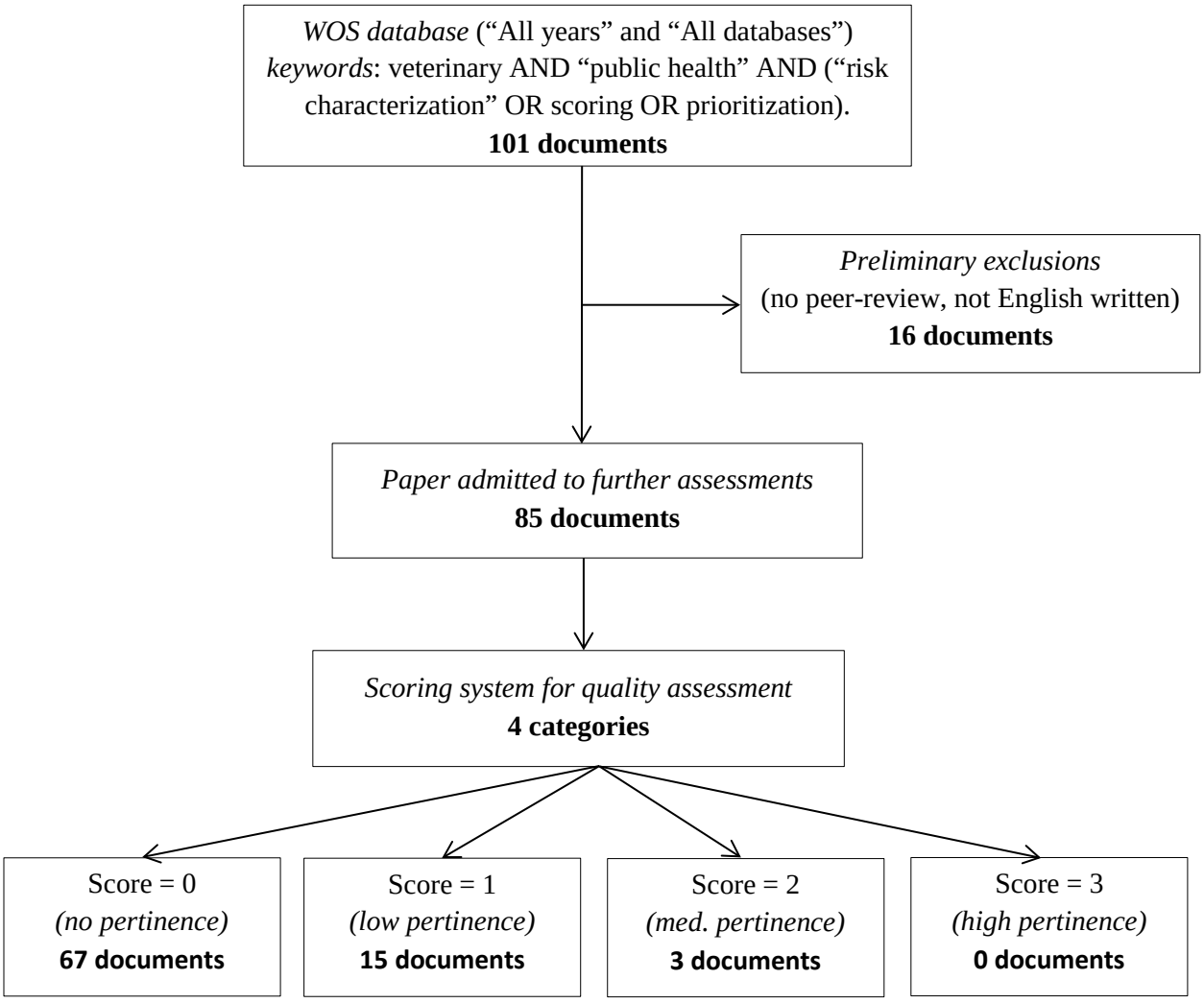

Figure 2 – Flowchart of the systematic review

3.4 Disease selection

The EP selected for assessment an initial group of 38 diseases of major interest in Lombardy region, classified by animal species affected, as summarized in Table 3. EP chose these disease for several reasons: they could be widespread, they may have a serious impact if introduced, or they are reportable diseases. Bacterial and viral infections represent the majority of the diseases selected, nonetheless, some protozoan, prion, and macroparasitic diseases were also included.

Bovine	**Small Ruminant**	**Swine**
Bovine brucellosis	Blue tongue	Aujeszky's disease
Bovine tuberculosis	Brucellosis (small rum.)	Classic swine fever
Bovine spongiform encephalopathy (BSE)	Contagious agalactia	Porcine reproductive and respiratory syndrome (PRRS)
Bovine viral diarrhoea (BVD)	Query Fever	Salmonellosis (swine)
Enzootic bovine leucosis	Transmissible spongiform encephalopathy (small rum. TSE)	Swine erysipelas
Infectious bovine rhinotracheitis (IBR)		Swine vesicular disease
Listeriosis		Trichinosis (swine)
Mastitis (*S. agalactiae*)		
Mastitis (*S. aureus*)		
Paratuberculosis		
Salmonellosis (bovine)		

Poultry	**Equine**	**Others**
Avian campylobacter	Equine infectious anaemia	Campylobacteriosis (pets)
Avian influenza	Equine viral arteritis	Leishmaniasis
Fowl typhoid	Trichinosis (equine)	Nosemosis
Newcastle disease		Opisthorchiasis
		Rabies
		Toxoplasmosis
		Varroosis
		West Nile fever

Table 3 – Diseases of interest in Lombardy, subdivided by species.

Specifically, 11 bovine disease were selected. Three of them are reportable diseases included in the region official control plan (bovine tuberculosis, bovine brucellosis, enzootic bovine leucosis); three are considered to be a major risk for human health (bovine spongiform encephalopathy, listeriosis, salmonellosis) and the remaining four were chosen because of their

economic impact and/or as possible threat for public health (bovine viral diarrhoea, infectious bovine rhinotracheitis, paratuberculosis, contagious mastitis).

All the five small ruminant diseases included present a relevant economic impact and/or pose a severe threat to animal health; furthermore, two of them are zoonosis (brucellosis, query fever).

Seven swine diseases were included, three of them are a risk for human heath (salmonellosis, swine erysipelas, trichinosis) while the other four may pose a relevant threat for swine health and/or economics (Aujeszky's disease, classic swine fever, swine vesicular disease, porcine reproductive and respiratory syndrome).

Four major poultry diseases were selected for assessment because of their economic relevance (fowl typhoid, Newcastle disease) and/or their negative impact on public health (avian influenza, avian campylobacter).

Three equine diseases were chosen, two of them are reportable diseases without zoonotic potential (equine infectious anaemia, equine viral arteritis) while the third (trichinosis) is also a severe threat for food safety.

Finally, eight animal diseases were included within the category "other"; these are disease of interest for public health that cannot fit within only one of the previous categories (campylobacteriosis in pets, leishmaniasis, rabies, toxoplasmosis, West Nile fever, opisthorchiasis) or they represent a major problem for beekeeping (nosemosis, varrosis).

3.5 Scoring process and data sources

The scores of the selected diseases were proposed by two experts, one from DIVET and one from VOL, and then discussed by the EP during plenary meetings. These two expert, alongside with an assistant (the same of the FCP or the facilitator), filled a very specific form (see the appendix for three examples) that had to contain all the information needed for a proper scoring of each category within the scorecard. If, during this phase, some pivotal information were missing the scoring process was stopped until these information became available. Finally, the preliminary scores were considered approved when at least 9 out 12 expert (consensus set at 75%) agreed on these ranks.

General and scientific information regarding the diseases under assessment came from peer-reviewed scientific papers and reviews, only documents published on academic journal with an impact factor (IF) reported on Journal Citation Reports (JCR) were considered. Additional reliable scientific information were selected from other sources, precisely:

- *Scientific books*: general scientific book on infectious diseases (e.g. Fenner's Veterinary Virology, IV Ed., edited by: N. J. Maclachlan and E. J. Dubovi, Academic Press, 2010, ISBN: 978-0-12-375158-4) or monographic publications about a specific disease (e.g. Paratuberculosis: Organism, Disease, Control, edited by M. A. Behr and D. M. Collins, CABI, 2010, ISBN: 978-1-84-593613-6).
- *World Organisation for Animal Health (OIE)*: Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (available at http://www.oie.int/international-standard-setting/terrestrial-manual/access-online/) and technical disease cards (available at http://www.oie.int/animal-health-in-the-world/technical-disease-cards/).
- *Center for Food Security and Public Health (CFSPH)* of Iowa State University: Diseases Technical Factsheet (available at http://www.cfsph.iastate.edu/DiseaseInfo/).
- *Other institutes and organisations*: publications by World Health Organization (WHO), Centers for Disease Control and Prevention (CDC), European Food Safety Authority (EFSA), European Centre for Disease Prevention and Control (ECDC), European Medicines Agency (EMA), Food and Agriculture Organization of the United Nations (FAO).

Various organisations reports and an international database were selected as source of epidemiological data such as:

- VOL official reports.
- Experimental Zooprophylactic Institutes reports.
- Italian national epidemiological bulletins.
- EFSA reports.
- ECDC reports.
- CDC reports.
- WHO reports.
- World Animal Health Information Database (WAHID).

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4 Results

Two main results were produced during this study: the scorecard model itself and the subsequent ranking of 23 of 38 diseases identified by the EP as diseases of particular interest at Regional-level.

4.1 Scorecard final model

The scorecard model, developed during the FCP, represents the first result of the study. This final model was also included and published in the VPP of Lombardy region.

The final model of the scorecard (Table 4) covers six areas of interest:

1. Disease relevance.
2. Socio-economic impact.
3. Impact on public health (human).
4. Impact on trade.
5. Impact on animal welfare.
6. Control tools.

Each area is composed of different categories. The number of categories within each area vary from a minimum of four (socio-economic impact, impact on trade, control tools) to a maximum of 11 (relevance of the disease).

The scoring system of the model and the categories of the various areas will be further described in the following sections.

SCORECARD								
Disease Name		**SCORE = 551**						
Criteria		**Score**					**Coef**	**Tot**
1	**Relevance Of The Disease**	**1**	**2**	**3**	**4**	**5**	**1.43**	**41.4**
1.1	Presence of the disease	1					2.86	
1.2	Frequency of the disease		2					
1.3	Number of species involved			3			4.29	
1.4	Speed of spread				4		5.71	
1.5	Vectors as reservoir and potential source of the disease					5	7.14	
1.6	Risk of spread to susceptible species				4		5.71	
1.7	Wildlife as reservoir and potential source of the disease			3			4.29	
1.8	Potential for silent spread		2				2.86	
1.9	Variability of the agent	1					1.43	
1.10	Knowledge of host-pathogen interaction		2				2.86	
1.11	Knowledge of immunology			3			4.29	
2	**Socio-Economic Impact**	**1**	**2**	**3**	**4**	**5**	**10.00**	**100**
2.1	Impact on production within the region	1					10.00	
2.2	Economic impact of the control plan		2				20.00	
2.3	Potential economic direct impact			3			30.00	
2.4	Potential economic indirect impact				4		40.00	
3	**Impact On Public Health**	**1**	**2**	**3**	**4**	**5**	**8.57**	**189**
3.1	Relevance in laws (locals to international)	1					8.57	
3.2	Zoonotic potential		2				17.14	
3.3	Likelihood of occurrence			3			25.71	
3.4	Spread in humans				4		34.29	
3.5	Impact on human health					5	42.86	
3.6	Impact on food safety				4		34.29	
3.7	Bioterrorism potential			3			25.71	
4	**Impact On Trade**	**1**	**2**	**3**	**4**	**5**	**10**	**100**
4.1	Impact on regional trade due to current laws	1					10	
4.2	Impact on national / EU trade due to current laws		2				20	
4.3	Impact on international trade due to current laws			3			30	
4.4	Potential for zoning				4		40	
5	**Impact On Animal Welfare**	**1**	**2**	**3**	**4**	**5**	**2.86**	**71**
5.1	Potential impact on animal welfare (duration)					5	57.14	
5.2	Potential frequency of severe distress				4			
5.3	Severity / reversibility of the disease			3			8.57	
5.4	Impact on animal freedom		2				5.71	
6	**Control Tools**	**1**	**2**	**3**	**4**	**5**	**5**	**50**
6.1	Proper tools for diagnosis	1					5	
6.2	Proper tools for prevention (within the region/area)		2				10	
6.3	Proper tools for control (within the region/area)			3			15	
6.4	Proper tools for therapy				4		20	

Table 4 – Scorecard model (scores are illustrative).

4.1.1 Scoring system

All categories may be scored from 1 (best case) to 5 (worst) and, in order to ensure an identical score within the same area, a correction factor is applied after the scoring ("coeff." in Table 4). Moreover, each area presents a coefficient of weight, from 1 to 3, based on the relevance of the area itself (Table 5); these coefficients of weight were established by the EP during the FCP.

The final score of the model is the sum of the scores of all areas and may reach and its theoretical maximum is 1,000. Furthermore, every area presents a criticality level (Table 5) expressed in percent and calculated as the ratio of its score and the highest score achievable and (maximum theoretical level 100%).

Microsoft® Excel 2010 (Microsoft Corporation, Redmond WA) was used to perform all the calculation.

Area of interest	Coefficient	Max. Weight	Crit. %
1. Relevance Of The Disease	1	100	Up to 100%
2. Socio-Economic Impact	2	200	Up to 100%
3. Impact On Public Health	3	300	Up to 100%
4. Impact On Trade	2	200	Up to 100%
5. Impact On Animal Welfare	1	100	Up to 100%
6. Control Tools	1	100	Up to 100%

Table 5 – Summary of areas of interest, weight and criticality levels. Criticality levels represents the percentage of maximum theoretical score that the disease can reaches in the related area of interest, based on its score in that area.

4.1.2 Relevance of the disease

"*Relevance of the disease*" has a coefficient of weight equal to 1 and 11 categories, this area interest focus on epidemiological and scientific data.

The most relevant categories are "*presence of the disease*" and "*frequency of the disease*". "*Presence of the disease*" analyses where the infection is reported (in the region, in Italy or in EU) and "*frequency of the disease*" assesses disease epidemiological pattern (from sporadically to endemic). The scores of these two categories are multiplied between themselves and provide over one-third of the final score of their area of interest.

The remaining categories examine scientific knowledge available regarding a given disease, specifically:

- Number of species involved
- Speed of spread
- Vectors as reservoir and potential source of the disease
- Risk of spread to susceptible species
- Wildlife as reservoir and potential source of the disease
- Potential for silent spread
- Variability of the agent
- Knowledge of host-pathogen interaction
- Knowledge of immunology

Table 6 illustrates the scoring criteria of the 11 categories related to "*relevance of the disease*".

4.1.3 Socio-economic impact

"*Socio-economic impact*" has a coefficient of 2 and 4 categories. Two categories, named "*impact on production within the region*" and "*economic impact of the control plan*", consider the actual impact of the diseases within the region. "*Impact on production within the region*" is represented by the current losses (production and quality) and "*economic impact of the control plan*" by the cost of the control plan (if present). The other two categories, "*potential economic direct impact*" and "*potential economic indirect impact*", assess the potential socio-economic impact of a disease (direct and indirect) in the worst-case scenario. "*potential economic direct impact*" analyses the cost of control (therapies, vaccination, culling, etc.) and the risks on production losses. "*Potential economic indirect impact*" examines potential market loss, impact of the disease on public health budget (human cases only), reduction of tourism, and threats to biodiversity. Table 7 reports the scoring criteria for the estimation of socio-economic impact.

Area of interest and Categories		Score				
1	**Relevance Of The Disease**	**1**	**2**	**3**	**4**	**5**
1.1	*Presence of the disease*	None	Present in EU / neighbour nations	Present in the nation / neighbour regions	Present in the Region	Endemic
1.2	*Frequency of the disease*	None	Low Occasional reports.	Never studied / Unknown	High Reservoirs present and stable (livestock, pet, wildlife or vectors).	Very high
1.3	*Number of species involved*	Never studied, likely limited.	One species.	Limited 2 species.	High 3 species.	Very high 4 species or more.
1.4	*Speed of spread*	None Not transmissible.	Very slow Low level of spread within the herd, unlikely between herds.	Slow Slow spread between herds only with animal movements.	Fast Spread between herds with or without animal movements.	Very Fast Fast spread between herds without animal movements.
1.5	*Vectors as reservoir and potential source of the disease*	None No biological / mechanical vectors	Low Presence of the vectors unknown, very unlikely within the country.	Medium Vectors present within the country but not considered capable of surviving and transmitting the disease.	High Vectors present within the country and able of transmitting the disease but not considered capable of surviving.	Very high Vectors are a stable presence within the country and capable of transmitting the disease.
1.6	*Risk of spread to susceptible species*	None Not contagious.	Low Spread only due to direct contact.	Unknown	High Spread also due to indirect contact.	Very High Airborne.
1.7	*Wildlife as reservoir and potential source of the disease*	None No wild animal *reservoir* / potential source of the disease.	Low Low prevalence in isolated wild animals.	Medium Wild animals *reservoir* but no direct contact with livestock, pets or humans.	High Wild animals reservoir, occasional contact with livestock, pets or humans.	Very High Wild animals *reservoir* in close contact with livestock, pets or humans
1.8	*Potential for silent spread*	None	Low Signs of infection easily recognised and likely to occur in animal under control.	Medium Signs of infection easily recognised but depends on level / plans of surveillance.	High Specific diagnosis may be difficult in one or more species.	Very High Disease/infection not likely to be detected for certain time.
1.9	*Variability of the agent*	None One type only, stable host/vector	Low Few types without mutation, stable host and vectors (if any)	Medium Few types without mutation but low host-specificity, stable vectors (if any).	High Numerous types or mutating, low host-specificity or vector - specificity.	Very High Numerous types and mutating, low host- or vector - specificity.
1.10	*Knowledge of host-pathogen interaction*	Complete knowledge of host-pathogen interaction.	Almost complete knowledge of host-pathogen interaction.	Partial knowledge of host-pathogen interaction.	Almost no knowledge of host-pathogen interaction.	No knowledge of host-pathogen interaction.
1.11	*Knowledge of immunology*	Complete knowledge of immunology (both humoral and cellular).	Complete knowledge of humoral immunity Partial knowledge of cellular immunity.	Partial knowledge of immunology (both humoral and cellular).	Partial knowledge of humoral immunity only.	No knowledge of immunology.

Table 6 – Relevance of the disease: categories and scoring criteria.

Area of interest and Categories		Score				
2	**Socio-Economic Impact**	**1**	**2**	**3**	**4**	**5**
2.1	*Impact on production within the region*	None Production is not touched.	Very low Some production losses but no serious impact on the income (< 5%).	Low Production losses < 20%. Qualitative drop of products is possible.	Medium Production losses > 20%. Qualitative drop of products.	High Production losses > 50% and Menace to survival of livestock industry.
2.2	*Potential economic direct impact*	None No restrictions or mandatory control measures.	Very low Restrictions on animal transfers or some production losses (< 5%).	Low Restrictions on production; mandatory vaccination (or similar control measures) or production losses < 20%.	Medium Restrictions on production; mandatory test and cull or production losses > 20%.	High Restrictions on production; mandatory stamping out or menace to survival of livestock industry.
2.3	*Potential economic indirect impact*	None No drop / restrictions on products distribution, tourism or biodiversity.	Very low Minor consequences on products distribution, tourism or biodiversity.	Low Herd products redirected to lower value markets. Possible damages on tourism or biodiversity	Medium Market price reduced temporarily by less than 30% . Relevant impact on public health budget. Damages on tourism or biodiversity.	High Market price reduced temporarily by more than 30% over a month or a country-wide ban. Important impact on public health budget, on tourism or biodiversity.
2.4	*Economic impact of the control plan*	None No control plan available.	Very low Voluntary control plan or surveillance	Low Mandatory control plan due to the free-status (only surveillance needed).	Medium Mandatory control with rare outbreaks (surveillance + outbreak closure).	High Mandatory control with numerous outbreaks (disease common in the region).

Table 7 – Socio-economic impact: categories and scoring criteria.

4.1.4 Impact on public health

"*Impact on public health*" has a coefficient of 3, 7 categories, and it represents the most important area of the scorecard. This area considers the relevance in the regional / national / international laws ("*relevance in laws*"). Moreover, "*Impact on public health*" includes "*zoonotic potential*" analyses the human-animal interface and the routes of spread between animals and humans. "*Likelihood of occurrence*" considers the incidence of a disease in the region. "*Spread in humans*" assesses the likelihood of transmission between humans. "*Impact on human health*" analyses the effects of a disease on human health such as severity of symptoms, permanent health damage, and fatality rate.

Area of interest and Categories		Score				
3	**Impact On Public Health**	**1**	**2**	**3**	**4**	**5**
3.1	*Relevance in laws (locals to international)*	None	Regional	National	EU Area	International
3.2	*Zoonotic potential*	None Transmission between animals and human is not possible.	Very low Transmission between animals and human is not known or no information available.	Low Transmission is possible due to direct contact with live animals.	Medium Transmission is possible due to direct / indirect contact with live animals, vectors or food.	High Very low species barrier, possible airborne transmission or through the environment.
3.3	*Likelihood of occurrence*	None Proven impossibility of transmission to humans through live animals, animal products, vectors or food.	Very low Probability lower than 1/1.000.000.	Low Probability lower than 1/100.000.	Medium Probability lower than 1/10.000.	High Probability higher than 1/1.000.
3.4	*Spread in humans*	None Transmission between humans is not possible.	Very low Transmission between humans is not known but very unlikely.	Low Transmission between humans is uncommon.	Medium Transmission between humans requires prolonged or high level challenge.	High Transmission between humans occurs frequently.
3.5	*Impact on human health*	None Humans are not considered susceptible to infection.	Very low Symptoms are mild, transitory without lasting effects.	Low Symptoms may require time off work (up to 1week) and/or medical intervention.	Medium Symptoms often provoke medical intervention, possible long term health effects (>1 month). Severe pain and discomfort. Fatalities are uncommon.	High High case fatality (>5%) and/or permanent health effects.
3.6	*Impact on food safety*	None Transmission via food is not possible.	Very low Very low level of contamination of food, unlikely to cause problems.	Low Low level of contamination that can cause disease/infection only if agents are ingested in large quantities.	Medium Medium probability of spread via food but large numbers of organisms needed to cause problems. Precaution measures required.	High High probability of spread via food with small infective dose and strict precautions required.
3.7	*Bioterrorism potential*	None Agent unavailable / impossible to handle or harmless to humans.	Very low Agent available but difficult to handle or low potential to harm humans.	Low Agent available and easy to handle by professionals and labs but with low potential to harm humans.	Medium Agent available and easy to handle by professionals and labs and with high potential to harm humans.	High Agent available and easy to handle by single individuals and with high potential to harm humans.

Table 8 – Impact on public health: categories and scoring criteria.

"*Impact on food safety*" reflects both the likely level of contamination in food and the infectious dose. Finally, "*bioterrorism potential*" evaluates the availability of the agent and its potential, if used as a biological weapon, to cause substantial harm. Table 8 reports the scoring criteria implemented for assess impact on public health of a given disease.

4.1.5 Impact on trade

"*Impact on trade*" has a coefficient of 2 and 4 categories. Three categories consider the impact of a disease in regional / EU / international trade according to the current legislation. Namely, "*impact on regional trade due to current laws*", "*impact on national / EU trade due to current laws*", and "*impact on international trade due to current laws*". Their scores are based on the restriction on trades if an outbreak occurs or if the disease is endemic; the risk of losing an "area-free" status (when present) and the difficulty to regain this status.

The fourth category, "*potential for zoning*", analyses the possibility to create restricted areas in order to control an outbreak (from the single positive farm to the entire Country). Table 9 illustrates the categories and the scoring criteria regarding the impact on trade of a disease.

Area of interest and Categories		**Score**				
4	**Impact On Trade**	**1**	**2**	**3**	**4**	**5**
4.1	*Impact on regional trade due to current laws*	None No restrictions or only at animal-level.	Low Restrictions only at heard- / farm-level.	Medium Restrictions at zone-level and/or list of banned commodities .	High Restrictions at zone-level and without list of banned commodities.	Very high Restrictions / ban at regional-level.
4.2	*Impact on national / EU trade due to current laws*	None No restrictions or only at animal-level.	Low Restrictions only at heard- / farm-level	Medium Restrictions at zone-level or list of banned products.	High Restrictions at zone-level with or without list of banned commodities.	Very high Restrictions / ban at national-level.
4.3	*Impact on international trade due to current laws*	None No restrictions or only at animal-level.	Low Restrictions only at heard- / farm-level or further restrictions only in small number of countries due to particularly strict laws.	Medium Restrictions at zone-level or list of banned products. No loss of "free-status". Strong restrictions in large number of countries due to national laws.	High Restrictions at zone-level and loss of "free-status" but short recovery period of the status.	Very high Restrictions / ban at national-level, loss of "free-status" with long recovery period.
4.4	*Potential for zoning*	Very High Zoning possible at heard / farm level	High Zoning possible within 10 kms (6 miles)	Medium Zoning possible but more than 10 kms needed.	Low Zoning possible due to using wider administrative boundaries	None Only compartments.

Table 9 – Impact On Trade: categories and scoring criteria.

4.1.6 Impact on animal welfare

"*Impact on animal welfare*" has a coefficient of 1 and 4 categories. Specifically, "*Potential impact on animal welfare (duration)*" evaluates the duration of negative effects on animal welfare induced by a diseases. "*Potential frequency of severe distress*" assesses the percentage of animal with severe distress during an outbreak. "*Severity / reversibility of the disease*" analyses the consequences of a disease on animal health, such as severity of symptoms and permanent health damage; therapy availability and success are also assessed.

The fourth category, "*impact on animal freedom*", considers the potential restriction on four out of the "Five Freedom". Indeed, "Freedom from Fear and Distress" was not included within the scoring criteria because it was not considered relevant to the purposes of the scorecards.

"*Impact on animal welfare (duration)"* and "*potential frequency of severe distress*" are multiplied between themselves and provide over two-third of the final score of this area.

Table 10 reports the scoring criteria of the 4 categories related to "*impact on animal welfare*".

Area of interest and Categories		Score				
5	**Impact On Animal Welfare**	**1**	**2**	**3**	**4**	**5**
5.1	*Potential impact on animal welfare (duration)*	None No impact.	Very low Less than 48 hours.	Low 48 hours - 14 days.	Medium 15 days - 24 months.	Permanent More than 24 months.
5.2	*Potential frequency of severe distress*	None No animal infected.	Very low <5% in severe distress.	Low 6-20% in severe distress.	Medium 21-50% in severe distress.	High >50 % in severe distress.
5.3	*Severity / reversibility of the disease*	Asymptomatic	Mild clinical signs, self-limiting or minimum therapy needed.	Moderate clinical signs and therapy needed.	Severe clinical signs with possible therapy failure and permanent health effects.	Severe clinical signs, no therapy available and permanent health effects.
5.4	*Impact on animal freedom*	No limitations on "Animal Freedoms" (Freedom from Fear and Distress NOT included).	Limitations on 1 Freedom (Freedom from Fear and Distress NOT included).	Limitations on 2 Freedoms (Freedom from Fear and Distress NOT included).	Limitations on 3 Freedoms (Freedom from Fear and Distress NOT included).	Limitations on 4 Freedoms (Freedom from Fear and Distress NOT included).

Table 10 – Impact On Animal Welfare: categories and scoring criteria.

4.1.7 Control tools

"*Control tools*" has a coefficient of 1 and 4 categories. The category "*Proper tools for diagnosis*" encompasses validated kits availability within the nation; laws that rule the surveillance; techniques described by international organisation (OIE, WHO, UE) and DIVA test availability in order to emit an overall judgement on these tools. Moreover, an overall judgement on both available tools for control and tools adopted within the region is required.

"*Proper tools for prevention*" and "*Proper tools for control*" categories examine obstacles, incentives, available approaches, and vaccination strategies (i.e. laws, availability and efficacy) with the purpose of assess the status of surveillance, prevention and control in the region.

Finally, "*Proper tools for therapy*" considers the presence of appropriate protocols for therapy (if permitted) and related legislation.

Table 11 illustrates the scoring criteria adopted for risk characterisation in the area of interest "*control tools*".

Area of interest and Categories		**Score**				
6	**Control Tools**	**1**	**2**	**3**	**4**	**5**
6.1	*Proper tools for diagnosis*	Very high	High	Medium	Low	None
6.2	*Proper tools for prevention (within the region/area)*	Very high	High	Medium	Low	None
6.3	*Proper tools for control (within the region/area)*	Very high	High	Medium	Low	None
6.4	*Proper tools for therapy*	Very high / Forbidden	High	Medium	Low	None

Table 11 – Control Tools: categories and scoring criteria.

4.2 Scores of the diseases

The scorecard model, once approved from the Regional health authorities, was used for assessing risk priorities on an initial number of diseases affecting different food-producing species and, in few cases, pet animals.

To date 23 diseases out of the 38 selected were evaluated and Table 17 reports total scores and criticality levels of these diseases. In addition, the detailed scores of each disease are reported in the next sub-chapters (see Table 13 to Table 16).

The 15 diseases present in Table 3 but not yet scored are under scrutiny, as an on-going process. Indeed, data available on these 15 remaining diseases were considered insufficient or unreliable, and thus, they are still under examination.

Table 12 reports an extract of the scorecard form about the area of interest "*controls tools*".

6	CONTROL TOOLS	
6.1	**Proper tools for diagnosis**	
6.1.1	*Validated kits availability within the nation*	To be filled
6.1.2	*Laws that rule the surveillance*	To be filled
6.1.3	*Techniques described by international organisation (OIE, WHO, UE, etc.)*	To be filled
6.1.4	*DIVA test possibility / obligation of use*	To be filled
6.1.5	*Overall judgement on tools for control*	To be filled
6.2	**Proper tools for prevention (within the region/area)**	
6.2.1	*Obstacles / incentives to prevention*	To be filled
6.2.2	*Available prevention strategies and their efficacy*	To be filled
6.2.3	*Commercial vaccines availability in EU / Worldwide*	To be filled
6.2.4	*Marker vaccines availability in EU / Worldwide*	To be filled
6.2.5	*Vaccination efficacy*	To be filled
6.2.6	*Laws that rule vaccination*	To be filled
6.3	**Proper tools for control (within the region/area)**	
6.3.1	*Obstacles / Incentives to control*	To be filled
6.3.2	*Available control strategies and their efficacy*	To be filled
6.3.3	*Laws that rule control strategy*	To be filled
6.4	**Proper tools for therapy**	
6.4.1	*Therapeutic protocol in use (cure and prophylaxis)*	To be filled
6.4.2	*Laws that rule therapies*	To be filled
6.4.3	*Residual risks / suspension time*	To be filled

Table 12 – Control Tools: scorecard form.

4.2.1 Categories and final scores: bovine diseases

Final scores, among the 10 bovine diseases evaluated, vary from 327 to 547. Specifically:

- Mastitis (*S. aureus*) (547)
- Salmonellosis (537)
- Paratuberculosis (528)
- Bovine brucellosis (524)
- Mastitis (*S. agalactiae*) (509)
- Bovine tuberculosis (507)
- Listeriosis (484)
- BVD (458)
- IBR (441)
- Enzootic bovine leucosis (327)

Table 13 reports the detailed scoring for each category of these diseases and their final score.

Categories	Mastitis (*S. aureus*)	Salmonellosis	Paratuberculosis	Bovine brucellosis	Mast. (*S. agalactiae*)	Bovine tuberculosis	Listeriosis	BVD	IBR	Enz. bov. leucosis
Relevance Of The Disease	**Score**									
Presence of the disease	4	5	5	3	4	3	5	5	4	3
Frequency of the disease	4	3	4	1	4	1	3	5	4	1
Number of species involved	2	5	5	5	2	5	5	5	2	2
Speed of spread	3	4	3	2	3	3	3	3	3	2
Vectors as reservoir and potential source of the disease	2	3	1	1	1	1	1	2	1	2
Risk of spread to susceptible species	4	5	3	4	4	4	2	5	5	2
Wildlife as reservoir and potential source of the disease	2	4	3	4	2	5	4	2	2	2
Potential for silent spread	5	4	5	4	5	4	4	5	3	4
Variability of the agent	5	5	3	4	4	4	4	5	3	3
Knowledge of host-pathogen interaction	3	2	3	2	3	2	3	2	2	2
Knowledge of immunology	3	2	3	2	3	1	2	2	1	2
Socio-Economic Impact	**Score**									
Impact on production within the region	4	2	3	1	4	2	2	4	3	1
Economic impact of the control plan	2	1	2	3	2	4	1	1	2	3
Potential economic direct impact	4	2	3	4	3	4	2	4	4	4
Potential economic indirect impact	2	3	2	4	2	3	2	2	2	1
Impact On Public Health	**Score**									
Relevance in laws (locals to international)	4	5	1	5	3	5	5	1	1	1
Zoonotic potential	4	5	2	4	4	4	4	1	1	1
Likelihood of occurrence	3	3	2	2	2	2	2	1	1	1
Spread in humans	2	3	1	4	3	3	3	1	1	1
Impact on human health	3	3	4	4	3	3	3	1	1	1
Impact on food safety	4	4	2	4	2	3	4	1	1	1
Bioterrorism potential	2	3	1	3	2	2	2	1	1	1
Impact On Trade	**Score**									
Impact on regional trade due to current laws	2	2	2	2	2	2	2	1	3	2
Impact on national / EU trade due to current laws	2	2	2	2	2	2	1	3	3	2
Impact on international trade due to current laws	1	2	2	2	1	2	1	2	3	2
Potential for zoning	1	1	5	1	1	1	1	5	1	1
Impact On Animal Welfare	**Score**									
Potential impact on animal welfare (duration)	4	3	5	4	4	4	3	4	3	4
Potential frequency of severe distress	3	2	2	2	3	2	2	2	4	2
Severity / reversibility of the disease	4	3	5	2	4	4	4	3	3	5
Impact on animal freedom	3	3	3	3	3	3	4	4	3	2
Control Tools	**Score**									
Proper tools for diagnosis	2	2	4	1	2	1	2	2	4	2
Proper tools for prevention (within the region/area)	4	4	4	2	4	2	4	4	4	2
Proper tools for control (within the region/area)	3	4	4	2	3	2	4	4	4	1
Proper tools for therapy	3	2	5	1	3	1	3	1	1	1
Final weighted score	**547**	**537**	**528**	**524**	**509**	**507**	**484**	**458**	**441**	**327**

Table 13 – Bovine diseases: partial and final scores.

4.2.2 Categories and final scores: small ruminants diseases

Final scores, among the four small ruminant diseases evaluated, vary from 401 to 551. Specifically:

- Query fever (551)
- Small ruminants brucellosis (524)
- Blue tongue (499)
- Contagious agalactia (401)

Table 14 reports the detailed scoring for each category of these diseases and their final score.

Categories	Query fever	Brucellosis (sm. rum.)	Blue tongue	Contagious agalactia
Relevance Of The Disease	**Score**			
Presence of the disease	4	3	3	4
Frequency of the disease	3	1	1	3
Number of species involved	5	5	4	3
Speed of spread	4	2	5	3
Vectors as reservoir and potential source of the disease	5	1	5	1
Risk of spread to susceptible species	5	4	5	4
Wildlife as reservoir and potential source of the disease	4	4	4	2
Potential for silent spread	4	4	3	4
Variability of the agent	5	4	4	4
Knowledge of host-pathogen interaction	4	2	2	3
Knowledge of immunology	3	2	2	2
Socio-Economic Impact	**Score**			
Impact on production within the region	2	1	1	2
Economic impact of the control plan	1	3	2	2
Potential economic direct impact	4	4	5	4
Potential economic indirect impact	3	4	4	1
Impact On Public Health	**Score**			
Relevance in laws (locals to international)	4	5	1	1
Zoonotic potential	5	4	1	1
Likelihood of occurrence	3	2	1	1
Spread in humans	3	4	1	1
Impact on human health	3	4	1	1
Impact on food safety	2	4	1	1
Bioterrorism potential	3	3	1	1
Impact On Trade	**Score**			
Impact on regional trade due to current laws	2	2	3	2
Impact on national / EU trade due to current laws	2	2	3	2
Impact on international trade due to current laws	2	2	3	2
Potential for zoning	2	1	4	1
Impact On Animal Welfare	**Score**			
Potential impact on animal welfare (duration)	2	4	3	4
Potential frequency of severe distress	2	2	5	4
Severity / reversibility of the disease	2	2	5	4
Impact on animal freedom	3	3	5	5
Control Tools	**Score**			
Proper tools for diagnosis	3	1	2	2
Proper tools for prevention (within the region/area)	5	2	4	3
Proper tools for control (within the region/area)	4	2	3	3
Proper tools for therapy	3	1	4	3
Final weighted score	**551**	**524**	**499**	**401**

Table 14 – Small ruminants diseases: partial and final scores.

4.2.3 Categories and final scores: swine diseases

Among the six swine diseases evaluated the scores vary from 359 to 615, precisely:

- Salmonellosis (615)
- Classic Swine Fever (424)
- Aujeszky's disease (414)
- Swine Erysipelas (406)
- Swine vesicular disease (403)
- PRRS (359)

Table 15 reports the detailed scoring for each category of these diseases and their final score.

Categories	Salmonellosis	Classic Swine Fever	Aujeszky's disease	Swine Erysipelas	Swine vesicular disease	PRRS
Relevance Of The Disease	**Score**					
Presence of the disease	4	1	5	4	3	4
Frequency of the disease	4	1	4	2	1	4
Number of species involved	5	2	5	5	2	2
Speed of spread	4	3	3	3	4	4
Vectors as reservoir and potential source of the disease	3	1	3	1	1	3
Risk of spread to susceptible species	5	5	3	4	5	5
Wildlife as reservoir and potential source of the disease	4	2	3	1	2	2
Potential for silent spread	4	2	4	3	3	3
Variability of the agent	5	2	2	4	3	4
Knowledge of host-pathogen interaction	2	2	1	2	2	2
Knowledge of immunology	2	2	1	2	2	1
Socio-Economic Impact	**Score**					
Impact on production within the region	3	1	2	3	1	4
Economic impact of the control plan	4	3	4	1	3	1
Potential economic direct impact	4	5	3	3	5	4
Potential economic indirect impact	3	2	1	1	2	1
Impact On Public Health	**Score**					
Relevance in laws (locals to international)	5	1	1	3	1	1
Zoonotic potential	4	1	1	3	1	1
Likelihood of occurrence	4	1	1	2	1	1
Spread in humans	3	1	1	1	1	1
Impact on human health	3	1	1	4	1	1
Impact on food safety	4	1	1	1	1	1
Bioterrorism potential	3	1	1	1	1	1
Impact On Trade	**Score**					
Impact on regional trade due to current laws	2	4	1	2	4	1
Impact on national / EU trade due to current laws	2	4	5	2	4	1
Impact on international trade due to current laws	2	4	5	2	4	1
Potential for zoning	1	2	1	1	2	1
Impact On Animal Welfare	**Score**					
Potential impact on animal welfare (duration)	3	3	3	4	3	4
Potential frequency of severe distress	3	5	2	2	2	3
Severity / reversibility of the disease	4	5	3	4	4	3
Impact on animal freedom	3	2	3	2	2	2
Control Tools	**Score**					
Proper tools for diagnosis	2	1	2	2	1	3
Proper tools for prevention (within the region/area)	4	1	2	2	1	3
Proper tools for control (within the region/area)	4	1	2	2	1	2
Proper tools for therapy	3	1	1	2	1	2
Final weighted score	**615**	**424**	**414**	**406**	**403**	**359**

Table 15 – Swine: partial and final scores.

4.2.4 Categories and final scores: other diseases

Three diseases of the “other” group were scored as follows:

- Toxoplasmosis (575)
- West Nile fever (498)
- Opisthorchiasis (444)

Table 16 reports the detailed scoring for each category of these diseases and their final score.

Categories	Toxoplasmosis	West Nile fever	Opisthorchiasis
Relevance Of The Disease	**Score**		
Presence of the disease	4	3	4
Frequency of the disease	3	2	3
Number of species involved	5	5	4
Speed of spread	4	3	2
Vectors as reservoir and potential source of the disease	2	5	5
Risk of spread to susceptible species	5	4	3
Wildlife as reservoir and potential source of the disease	4	5	3
Potential for silent spread	5	4	5
Variability of the agent	2	3	2
Knowledge of host-pathogen interaction	1	3	2
Knowledge of immunology	1	3	3
Socio-Economic Impact	**Score**		
Impact on production within the region	2	3	3
Economic impact of the control plan	1	2	2
Potential economic direct impact	1	1	2
Potential economic indirect impact	2	1	2
Impact On Public Health	**Score**		
Relevance in laws (locals to international)	5	5	4
Zoonotic potential	5	5	4
Likelihood of occurrence	5	3	3
Spread in humans	4	3	1
Impact on human health	3	4	3
Impact on food safety	5	1	5
Bioterrorism potential	1	1	1
Impact On Trade	**Score**		
Impact on regional trade due to current laws	1	1	1
Impact on national / EU trade due to current laws	1	1	1
Impact on international trade due to current laws	1	1	1
Potential for zoning	5	4	3
Impact On Animal Welfare	**Score**		
Potential impact on animal welfare (duration)	3	3	1
Potential frequency of severe distress	3	3	1
Severity / reversibility of the disease	5	5	1
Impact on animal freedom	4	2	1
Control Tools	**Score**		
Proper tools for diagnosis	4	4	2
Proper tools for prevention (within the region/area)	4	2	4
Proper tools for control (within the region/area)	4	3	3
Proper tools for therapy	5	4	1
Final weighted score	**575**	**498**	**444**

Table 16 – Other diseases: partial and final scores.

4.2.5 Scores summary and criticality levels

All the final scores are summarised in Table 17. In addition, Table 17 reports criticality level for each area of interest of the assessed diseases. Indeed, every disease presents, at area-level, a criticality level calculated as the ratio of its score in that area and the highest score achievable (see Table 5). Criticality level are expressed in percent and are not influenced by the coefficient of weight of their area (see Table 5).

The scorecards and the fulfilled forms of the 23 diseases in Table 17 are available (in Italian) on the Regional Veterinary Office website with access restricted to the Regional Veterinary Service officers.

Three examples (bovine mastitis by *S. aureus*, bovine tuberculosis, bluetongue) of filled scorecards and their forms are reported in to the appendixes of this thesis.

Disease	Final Score	Relevance Of The Disease	Socio-Economic Impact	Impact On Public Health	Impact On Trade	Impact On Animal Welfare	Control Tools
Bovine							
Mastitis (*S. aureus*)	**547**	64%	60%	63%	30%	54%	60%
Salmonellosis	**537**	70%	40%	74%	35%	34%	60%
Paratuberculosis	**528**	70%	50%	37%	55%	51%	85%
Bovine brucellosis	**524**	44%	60%	74%	35%	37%	30%
Mastitis (*S. agalactiae*)	**509**	61%	55%	54%	30%	54%	60%
Bovine tuberculosis	**507**	46%	65%	63%	35%	43%	30%
Listeriosis	**484**	61%	35%	66%	25%	40%	65%
BVD	**458**	80%	55%	20%	55%	43%	55%
IBR	**441**	54%	55%	20%	50%	51%	65%
Enzootic bov. leucosis	**327**	34%	45%	20%	35%	43%	30%
Small ruminants							
Query fever	**551**	73%	50%	66%	40%	26%	75%
Brucellosis (small rum.)	**524**	44%	60%	74%	35%	37%	30%
Blue tongue	**499**	53%	60%	20%	65%	71%	65%
Contagious agalactia	**401**	54%	45%	20%	35%	71%	55%
Swine							
Salmonellosis	**615**	71%	70%	74%	35%	46%	65%
Classic Swine Fever	**424**	31%	55%	20%	70%	63%	20%
Aujeszky's disease	**414**	64%	50%	20%	60%	34%	35%
Swine Erysipelas	**406**	47%	40%	43%	35%	40%	40%
Swine vesicular disease	**403**	39%	55%	20%	70%	34%	20%
PRRS	**359**	60%	50%	20%	20%	49%	50%
Others							
Toxoplasmosis	**575**	59%	30%	80%	40%	51%	85%
West Nile fever	**498**	59%	35%	63%	35%	46%	65%
Opisthorchiasis	**444**	56%	45%	60%	30%	9%	50%

Table 17 – Final scores and criticality levels of the 23 assessed diseases. The colouration of criticality levels varies from green (lowest level) to red (highest level) and it is meant to facilitate the consultation of the table

5 Discussion

5.1 Methodological approach

When we started our study, we had to select a methodological approach suitable for our aims. Our projected was part of a joint initiative, between VOL and DIVET, designed to improve efficiency, efficacy and quality of the regional veterinary services. Hence, the choice of the method was influenced by available resources (human and financial) and a fixed timeline (the final model had to be ready in about one year).

We identified three approaches as potentially suitable for our objectives: consensus process, MCDA, and expert framework. An overview of the applications of these approaches in veterinary public health is reported in chapters 1.3 and 1.4.

MCDA included a large number of techniques to develop models for the evaluation of different alternatives. These models could provide an evaluation system for the raking or the selection of the best alternatives and they were applicable for several problems, practices, interventions, and solutions. In addition, MCDA was applicable even when uncertainties were present such as future consequences of interventions (41), difficulties in problem identification (42), and limited availability of precise information (43).

During the last few years, MCDA has seen an increasing popularity in healthcare and public health. Indeed, more than 50% of the papers about MCDA in healthcare were published since 2011, as reviewed in (44). However, these models focused only on human health and when we started our project there was only one published paper on the application of MCDA in veterinary public health. This study adopted MCDA to evaluate control strategies for classical swine fever within the EU (16). Recently, two interesting papers were published on the topic: one regarding the ranking of emerging threats to animal health in UK (45) and the other on the emergence of Lyme disease in Quebec (13).

We decided to use an approach different from MCDA because we aimed to build a scoring model for the evaluation of very distinct areas of interest and each of these areas involved several uncertainties. Therefore, the development of a MCDA model in this background was not compatible with our resources. Precisely, we did not have enough human resources specialised in MCDA nor the time to develop this expertise because of our timeline.

Over the last decade, different expert frameworks developed various scoring model for setting priority in veterinary public health (17). Among these model, the DP scoring system represented the most useful source for our study due to its assessment of animal diseases in very distinct sector.

However, we decided to not use the expert panel approach because it did not include a specific method to follow *a priori* and it had required the development of specific guidelines. Therefore, we considered our resources too limited for this approach. For example, DP involved more 360 experts from 35 countries, 52 expert groups, and an investment of over 1 million € (46, 47).

Therefore, we identified the consensus process as the most suitable approach for our purposes. Indeed, these processes were based on consolidated guidelines and they were able to provide effective methods when scientific data is limited (12). The Delphi protocol represented the most applied consensus process. However, this protocol required anonymity in order to avoid dominant positions among the experts. Nonetheless, several member of our EP worked together or known each other, and thus, we considered the anonymity prerequisite difficult to satisfy. Hence, our final choice was FCP, a recently developed consensus process focused on healthcare and public health (27). After the setting of the final version of our model, the scorecards and their forms were proposed by two experts due to our strict deadlines. However, these forms and scores had to be approved by EP with, at least, the same degree of consensus (75%) adopted during the FCP.

5.2 Development of the model and scoring criteria

Tools for risk characterisation and prioritization should be based on scientific evidences. Nonetheless, these evidences are not always adequate, particularly in veterinary public health, and lack of specific data (or scientific papers) may undermine the development of a proper model. In conditions where the scientific evidence is scarce, consensus methods should be used to achieve agreement among experts (12). Indeed, we encountered two main obstacles during the development of our model. Namely, to find proper sources of information and to reach consensus within the working group, comparable difficulties were also reported in similar studies (48).

Our first step to find suitable sources of information was a systematic review (see chapter 3.3 and Appendix D). However, the results of this review were underwhelming and none of the included papers reached, after the quality assessment, the maximum rank.

Several models have been developed to prioritize disease on a broad scale (17), but none of them addressed scenarios involving specific areas and economic impact on a regional level, and thus, they provided an useful but limited source of information. In addition, different approaches and models for setting priorities in some fields of veterinary science, zoonosis, and food safety were proposed during the past years (12, 21, 32, 33, 48-50). Nevertheless, we did not find these models properly fit for the purposes of our project, with the exception of the recent DP model (35).

The DP model was an instrumental source of information because it facilitated identification and balance of both areas and categories in our scorecard. However, it should be emphasized that even if some diseases considered in DP and in our project are the same, the scorecards developed in in the two projects are different due to the different aims of the two projects. The scoring criteria and various categories are also different, and thus, a direct comparison between the scores of the diseases assessed by the two model is not feasible.

We adopted the FCP for the development our model because it provided a solid method to decide which and how the above mentioned sources had to be used and to overcome the limitations caused by lacks of information. Nonetheless, consensus processes are time-consuming approaches and during the FCP we encountered the second main obstacle of this study. Namely, the reaching of consensus among the experts on some of the addressed topics. These sources of disagreement will be further discussed in the following paragraphs.

The weight of the areas represented one of the main sources of conflict. Although the first draft and the final version of our scorecard included the same areas, the areas in the draft contributed equally to the total score of a disease (1/6 each). However, the focus of our model was primarily public health, and thus, some areas had to be more important than others. We identified "*impact on public health*" as the most important area. In addition, we recognised the vital importance of agro-food production in Lombardy, and so, the relevance of "*socio-economic impact*" and "*impact on trade*". After setting these priorities, EP established a coefficient of three for "*impact on public health*" and a coefficient of two for both "*socio-economic impact*" and "*impact on trade*". Nevertheless, is important to remark that the other areas ("*disease relevance*", "*impact on animal welfare*", "*control tools*") should not be underestimated. Contrary to the weight of the areas, both the identification of the disease of interest and their grouping, based on species, were not a significant source of conflict. "*Relevance of the disease*" was one of the simplest areas of interest to develop and EP did not encounter relevant obstacles to reach the consensus. Certainly, it represented the area with the greatest number of scientific sources available, and we selected its 11 categories to encompass epidemiological and scientific data available on a given diseases (see Table 6). Furthermore, the DP model provided a remarkable starting point for this area of interest.

The consensus process was slightly slowed-down only when the categories "*presence of the disease*" and "*frequency of the disease*" were discussed. Indeed, our goal was to develop a tool suitable at Regional-level and we identified these two categories as the most important of the entire area. In order to weight the two above mentioned categories we decided to multiply their scores between themselves, in this way, "*presence of the disease*" and "*frequency of the disease*" provided over one-third of the score of their area.

During the development of "*socio-economic impact*" area we pursued two objectives, namely, to analyse the actual economic impact of a disease in Lombardy and to estimate the potential impact of a disease in the worst-cases scenario (see Table 7).

We recognised, as an actual impact of a disease, not only the losses of production (qualitative and quantitative) caused by this disease but also the cost of a mandatory control plan (if present in the Region). EP did not encountered major problems of consensus during the definition of the two categories that assess the actual impact of a disease in Lombardy. Specifically, "*impact on production*" and "*economic impact of the control plan*".

We identified two categories for the estimation of the potential impact of a disease: one focused on the direct impact and the other on the indirect impact. We developed the category "*potential economic direct impact*" to estimate the direct costs of the introduction of a new disease in the Region or the possible consequences of a present disease if left uncontrolled. This category was not a relevant source of conflict within the EP. On the other hand, defining the criteria to estimate indirect costs was more problematic.

The first criterion identified for indirect impact was the potential price drop of food industry commodities. Indeed, in case of an outbreak of a zoonosis, fear and misinformation lead by *mass media* may cause relevant reduction of the market value. We also recognised the expenses for human treatments as potential source of indirect economic costs. For example, foodborne diseases from six agents (*Salmonella, Campylobacter jejuni, Escherichia coli* O157:H7, *Listeria monocytogenes, Staphylococcus aureus, Clostridium perfringens*) account for $2.9 – $6.7 billion in human health costs in the US each year (51). In addition, some experts proposed to comprise the impacts on tourism and on biodiversity because they both represented important resources for Lombardy region. In conclusion, EP reached consensus on the annexation of all the four above-mentioned criteria.

The evaluation of the category "*Potential economic indirect impact"* is heavily based on expert opinions (with the exception of human costs), and thus, particular carefulness should be employed during the scoring of this category. Furthermore, a new type of *mass media* will be encompassed in the future updates of the scorecards. Indeed, social networks has seen a massive increase of popularity during the last few years and these networks can now represent a concerning source of fear and misinformation, as recently happened with the ongoing outbreaks of Ebola virus disease.

The development of the area "*impact on public health*" did not encounter great obstacles. The only two categories that will be briefly discussed are "*relevance in laws (locals to international)*" and "*bioterrorism potential*". The other categories were based on epidemiology and they were already present in previous models (33, 34).

We included the category "*relevance in laws (locals to international)*" in order to contextualise the relevance of a zoonosis within the international laws, from a veterinary public health point of view. For example, bovine tuberculosis is included in both the OIE-Listed diseases (52) and the OIE Terrestrial Animal Health Code (53), while, swine erysipelas is contemplated only in at national-level (*Regolamento di Polizia Veterinaria, DPR 8 febbraio 1954, n. 320*).

The category "*bioterrorism potential*" in based on previous models (33, 34) and, in this case, the source of disagreement was if this category could be included or not within our scorecard. Finally, EP decided to include the category "*bioterrorism potential*" due to the large presence of potential sources of infectious agents (farm animals) and because the distribution of the population within Lombardy could facilitate the spread of an agent (large number of people in relatively narrow spaces).

The area of interest "*impact on trade*" was settled to assess the restrictions due to an outbreak (present or potential) and it considered laws at various geographical levels such as regional, national / EU, and worldwide. This area is based on the current laws and EP did not find noteworthy obstacles to consensus.

We encompassed the extent of the restrictions (from single animal to regional-wide), the potential loss of free-status, states with particularly strict laws, and possible ban of commodities. In addition, we added a category, "*potential for zoning*", that specifically addressed the size of the zone under restriction (see Table 9) because the possibilities of zoning directly influenced trade restrictions.

We identified four categories for the area "*impact on animal welfare*" and we considered this area important for veterinary public health, although it did not represent one of the major focus of our model. The four categories of the area (see Table 10) had the same importance in the draft. Nonetheless, our main purpose was to highlight how a disease could pose a potential threat for welfare. We recognised the categories "*potential impact on animal welfare (duration)*" and "*potential frequency of severe distress*" as the two most appropriate for our purpose. Thus, EP agreed on multiplying the scores of these two categories between themselves so they could provide over two-third of the final score of the area. Finally, EP decided to not include the evaluation of the "Freedom from Fear and Distress" within the category "*impact on animal freedom*" because this Freedom was difficult to evaluate, and thus, it could complicate the model without providing significant benefits. In addition, several instruments are already available (49, 54-61) to further assess animal welfare.

The last area, "*control tools*", was developed to analyse the current status of prevention and control in the region and to assess the general availability of proper instruments for diagnosis and therapy.

We selected quite simple categories and scoring criteria (see Table 11) and we focused more on the related form (see Table 12). EP did not found relevant source of disagreement during the development of this part of the form. Nonetheless, the sections of the form on prevention and control in the region require to assess available strategies and their efficacy, to identify obstacles and incentives, and an overall judgement on tools for control. Hence, these sections are strongly influenced by expert opinions and particular attention should be posed during the scoring process.

5.3 Scoring process and ranking of the diseases

When scorecard forms were to be filled, lacks of data were manifest for some of the diseases under assessment. These deficiencies were particularly noticeable when frequency of the disease and categories encompassing costs were considered. Shortages of information were slowing down the process of filling forms, and lacks of pivotal data explain why not all the diseases in Table 3 have, to date, a completed scorecard. Nonetheless, the discover of these deficiencies also represents an important opportunity to identify and address gaps within surveillance or control plans.

Final and partial scores of the 23 diseases analysed to date are based on 15 to 25 pages of forms with the collected information (see Appendix A, Appendix B, and Appendix C for three examples). Hence, the description and the discussion of scorecards for every single disease are not possible in this thesis. Nevertheless, some of the final scores (see Table 17) were unexpected and they deserve a brief discussion.

During the ranking of bovine diseases, classical zoonosis, e.g. bovine tuberculosis, received lower scores when compared to other diseases such as *S. aureus* mastitis. Although these ranks may seem surprising, they confirm the usefulness of the model when applied in a specific area. Indeed, the relatively low score of bovine tuberculosis is due to the disease free-status of Lombardy, while *S. aureus* mastitis is highly prevalent and these infections, even if they are not considered to have a significant zoonotic potential, represent a possible source of exposure to methicillin-resistant *Staphylococcus aureus* (MRSA) strains (62, 63). *S. aureus* infections are currently a matter of concern for public health because of the ability of *S. aureus* to evade immune defences (64-67) and the increasing diffusion of MRSA in both human and domestic animal (62, 63, 68-71). Furthermore, *S. aureus* can produce enterotoxins that may lead to food poisoning even after thermal processes (72, 73). Therefore, the risk of human exposure is potentially high and the specific scorecard, undoubtedly, emphasize the potential risks for public health in Lombardy, in contrast to the common belief that these problems are confined only to dairy herds.

In case of small ruminants diseases, query fever reached a higher score than brucellosis (551 versus 524, see Table 14 and Table 17) mainly because Lombardy is currently free of small ruminant brucellosis, while, query fever is present and proper plans for prevention or control are not available in the Region. In addition, the zoonotic potential of query fever is greatly enhanced by its primarily route of transmission, indeed, humans generally became infected through air-borne transmission (74).

The rank of swine diseases did not highlight unattended results. Nonetheless, the negative economic impact of PRRS on pig industry can be dramatic (75). This disease should not be underestimated, even if is not a threat for human health or current trades, and its score should be revised frequently.

The scoring process of the diseases within the group "other" is still at an early phase with only three out of eight diseases ranked.

5.4 Final remarks: limits of the scoring model

We believe that FCP was the most suitable method available for the aims study. Nonetheless, this process (and consensus processes in general) has its limitations. Therefore, in this chapter we will discuss the major limits of our model.

The scorecard model is an instrument developed for professionals of our field, especially veterinary officers, and the scorecards and the related information can be misinterpreted by untrained people. In particular, the areas "*impact on public health*" and "*socio-economic impact*" may contain sensitive data (e.g. impact on food safety, bioterrorism potential, economic impact of the control plan) that may not be prone to cherry-picking or misapprehensions. "*Impact on animal welfare*" may also represent a source of misinterpretation. Although these outcomes are unlikely, we decided to publish the scorecards on the platform *VetinWeb* with access restricted to the Regional Veterinary Service officers.

Another relevant limit, that may arise in future, is about the update of the scorecards. Indeed, our model is a dynamic tool that requires regulars updates, thus, the forms and the scores should be reviewed at least every three year. In addition, unscheduled updates should be made every time a major change in the disease background happens (e.g. gain/loss of official free-status, epidemics, breakthroughs in control strategies, etc.). However, the speed of the updates may be impaired by limited resources, both human and economic.

The last major limitation concerns the availability of data and information to fulfil the forms, and thus, to assign proper scores. As we already reported in chapter 5.3, some diseases are still under review because of data deficiencies. The most frequent sources of these uncertainties are epidemiological data or economic costs of diseases. Nonetheless, the discover of these gaps also represents a remarkable opportunity to address targeted researches. Hence, these studies may be aimed to identify significant weakness in risk management, surveillance and control plans.

6 Conclusions

Prioritization tools are becoming increasingly important in developing health policies and strategies in preventive human and animal medicine and food safety. These tools are available to assess and control important food safety aspects such as contamination during food processing, but there are very few focused on animal diseases and their impact on human health and food safety. Among the few models available, none of them is applicable in a definite geographical area comparable to Lombardy because they should fit not only with the disease epidemiological pattern but also with the specific socio-economic characteristics of the area. The tool we developed tries to fill these gaps encompassing not only diseases with zoonotic and food poisoning potentials but also animal infections with an alarming economic impact (real or potential).

The scorecard model proposed represents the results of both veterinary researchers and veterinary officers' expertise in veterinary public health and it is conceived to fit the Lombardy Region characteristics but, with minor modifications, it can be applied in similar geographical or political areas.

Within this framework, areas of interest and related categories were built to be as much objective as possible and the scoring criterion was developed to be clear and easy to understand for professional involved in the process. However, it is important to emphasize that the objective of our scorecard model is not to provide a sterile score system about a given disease. Indeed, we developed this scoring model as a dynamic tool to help professionals in prioritization and decision-making in very distinct sector. Furthermore, our model is based on scientific evidences and a firm consensus protocol in order to support prioritisation in risk management and to avoid decisions based on cognitive bias or on the pressures of public opinion's fears and misinformation driven by *mass media*.

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Appendix A: Bovine Tuberculosis

SCORECARD [last update 02-22-2012]								
BOVINE TUBERCULOSIS		**SCORE = 507**						
Criteria		**Score**					**Coef**	**Total**
1	**Relevance Of The Disease**	**1**	**2**	**3**	**4**	**5**	**1.43**	**45.7**
1.1	Presence of the disease			3			4.29	
1.2	Frequency of the disease	1						
1.3	Number of species involved					5	7.14	
1.4	Speed of spread			3			4.29	
1.5	Vectors as reservoir and potential source of the disease	1					1.43	
1.6	Risk of spread to susceptible species				4		5.71	
1.7	Wildlife as reservoir and potential source of the disease					5	7.14	
1.8	Potential for silent spread				4		5.71	
1.9	Variability of the agent				4		5.71	
1.10	Knowledge of host-pathogen interaction		2				2.86	
1.11	Knowledge of immunology	1					1.43	
2	**Socio-Economic Impact**	**1**	**2**	**3**	**4**	**5**	**10.00**	**130**
2.1	Impact on production within the region		2				20.00	
2.2	Economic impact of the control plan				4		40.00	
2.3	Potential economic direct impact				4		40.00	
2.4	Potential economic indirect impact			3			30.00	
3	**Impact On Public Health**	**1**	**2**	**3**	**4**	**5**	**8.57**	**189**
3.1	Relevance in laws (locals to international)					5	42.86	
3.2	Zoonotic potential				4		34.29	
3.3	Likelihood of occurrence		2				17.14	
3.4	Spread in humans			3			25.71	
3.5	Impact on human health			3			25.71	
3.6	Impact on food safety			3			25.71	
3.7	Bioterrorism potential		2				17.14	
4	**Impact On Trade**	**1**	**2**	**3**	**4**	**5**	**10**	**70**
4.1	Impact on regional trade due to current laws		2				20	
4.2	Impact on national / EU trade due to current laws		2				20	
4.3	Impact on international trade due to current laws		2				20	
4.4	Potential for zoning	1					10	
5	**Impact On Animal Welfare**	**1**	**2**	**3**	**4**	**5**	**2.86**	**43**
5.1	Potential impact on animal welfare (duration)				4		22.86	
5.2	Potential frequency of severe distress		2					
5.3	Severity / reversibility of the disease				4		11.43	
5.4	Impact on animal freedom			3			8.57	
6	**Control Tools**	**1**	**2**	**3**	**4**	**5**	**5**	**30**
6.1	Proper tools for diagnosis	1					5	
6.2	Proper tools for prevention (within the region/area)		2				10	
6.3	Proper tools for control (within the region/area)		2				10	
6.4	Proper tools for therapy	1					5	

	Summary	Crit.	Coeff	Weight
1	Relevance Of The Disease	46%	1	100
2	Socio-Economic Impact	65%	2	200
3	Impact On Public Health	63%	3	300
4	Impact On Trade	35%	2	200
5	Impact On Animal Welfare	43%	1	100
6	Control Tools	30%	1	100

	SCORECARD FORM [last update 12-02-2012]
Extra	**Disease**
E.1.1	*Name of the disease*
	Bovine tuberculosis
E.1.2	*Aetiological agent/s*
	Mycobacterium bovis. Acid-fast (Ziehl–Neelsen stain), Gram positive, aerobic, coccus of the Mycobacterium tuberculosis complex. This complex includes *M. tuberculosis*, *M. caprae* (also common cause of bovine tuberculosis in Eastern Europe), *M. africanum*, *M. canetti*, *M. microti*, and *M. pinnepedii*.
E.1.3	*Brief description*
	Diseases that can affect bovine and several other animal species (human included). Airborne transmission represents the most common cause of contagion in bovine, however, the infection may also occur via ingestion (foodborne) or, rarely, through wound contaminations. Clinical signs and anatomopathological lesions may vary greatly based on route of transmission, infectious dose, virulence of the strain, and resistance of the host. Symptomatology is variable but often includes weakness, weight loss, anorexia, and respiratory signs. Bovine tuberculosis is a zoonosis and, in humans, ingestion of infected raw milk represents the most frequent route of transmission.
1	**RELEVANCE OF THE DISEASE**
1.1 **1.2**	**Presence and frequency of the disease within the region / outside the region**
1.1.1	*Presence and frequency of the disease within the region*
1.2.1	None. Lombardy is officially free (sporadic outbreaks).
1.1.2	*Presence and frequency of the disease within near region / country*
1.2.2	Italy – Piedmont, Valle d'Aosta, Liguria, and all the region of centre-south of Italy (islands included). Europe – Several Countries such as Spain, Portugal, Greece, England, Ireland, Wales, Hungary, Romania, Latvia, and Lithuania.
1.1.3	*Frequency of epidemics (specify where)*
1.2.3	Not applicable.
1.1.4	*Animal Hosts / Vectors / Environment as source of disease*
1.2.4	Bovine represents both the natural host and the main reservoir of the diseases, however, some wild species may also act as reservoir for the infection. *M. bovis* is able to survive in the environment for long times, and thus, environment can be a possible source of disease.
1.1.5	*Seasonal cycles / outbreak influenced by climate anomalies*
1.2.5	Not applicable.

1.1.6	*Factors that facilitate the presence of the agent (scarce hygiene, biosafety, management, etc.)*
1.2.6	Factors that facilitate the presence of the agent vary depending on the productive system (i.e. industrialized countries vs. developing countries). In developed countries these factors are: trade of infected bovines (inadequate surveillance), scarce control of bovine tuberculosis in wildlife, poor biosecurity, and management errors (overcrowding of susceptible hosts within areas at risk).
1.1.7	*Stability of the agent within the environment*
1.2.7	Medium to high. *M. bovis* is able to survive in the environment for years if the conditions are adequate (low temperature, high humidity, no direct exposure to the sun). In addition, *M. bovis* has a moderate resistance to drying (conflicting data about resistance to high temperatures).
1.1.8	*Likelihood of eradication*
1.2.8	Unlikely (possible in domestic species at farm-, region- or country-level). Eradication in wildlife may be impossible. Moreover, the epidemiological role of environment as source of disease need further clarifications.
1.3	**Number of species involved**
1.3.1	*Number of species involved (specify which ones)*
	High. Bovines, sheep, goats, cervids, equines, swine, buffaloes, bison, Canidae, felines, and rodents.
1.4	**Speed of spread**
1.4.1	*Speed of spread within the farm*
	Medium to low (depending on density and overcrowding).
1.4.2	*Speed of spread between farms*
	Low (speed of spread may be slightly speeded-up in case of overcrowding and poor biosecurity). For example, in UK prevalence increased only from 1.3% (in 1996) to 3.5% (in 2003) despite the absence of restrictions and controls.
1.4.3	*Likelihood of spread without hosts movement outside the farm*
	Unlikely. Airborne transmission requires close contact between animals.
1.5	**Vectors as *reservoir* and potential source of the disease**
1.5.1	*Disease cycle influenced by vectors*
	No.
1.5.2	*Presence of the vectors within the regional / national territory*
	Not applicable.
1.5.3	*Presence of the vectors influenced by peculiar areas / climates*
	Not applicable.
1.5.4	*Vectors likelihood of survival, reproduction, spread the disease, act as reservoir*
	Not applicable.
1.6	**Risk of spread to susceptible species**
1.6.1	*Likelihood of transmission*
	Medium. Likelihood of transmission may vary based on density and overcrowding.
1.6.2	*Route of transmission*
	M. bovis spreads mainly through expectoration, milk, or faeces. In addition, infection via urine, semen, or vaginal secretions is possible but not common. Transmission can be airborne, foodborne, or through infected wounds. The importance of these routes of transmission can vary depending on the species involved. Vertical transmission is also possible but very rare.

1.6.3	*Peculiar condition that influence transmission*
	Overcrowding and close contacts between contagious animals and sensible hosts (airborne transmission). Infected milk administered to calves (foodborne transmission). Frequent interactions between infected wildlife and sensitive domestic species.
1.7	**Wildlife as reservoir and potential source of the disease**
1.7.1	*Species involved*
	High. Cervids, *Mustelidae*, wild boars, rodents, *Canidae*, felines. In addition, several exotic species such as: buffaloes, bison, primates, American camelids, kudus, tapirs, elks, elephants, rhinoceroses, possums, etc..
1.7.2	*wildlife / livestock and pet / human interactions*
	Wildlife can represent a source of bovine tuberculosis if contagious wild animals share areas with sensitive domestic species and humans. Deer can play an important role as reservoirs of the diseases in wildlife. Badgers seem to be an important source of infection in England and Ireland, they may transmit the diseases through several routes (bites and urines included) and, during the terminal phase of the infection, they seem to expel *M. bovis* in large doses. Wild boars are usually spillover hosts, however, in peculiar conditions (high density, favourable habitat and poor management) they may act as reservoir.
1.7.3	*Endangered species involved*
	Some Spanish Authors has indicated the disease as possible threat for lynxes.
1.8	**Potential for silent spread**
1.8.1	*Likelihood of recognition due to the clinical symptoms*
	Low. Symptoms are usually not specific and manifest during the terminal phases of the disease.
1.8.2	*Spread by subclinical / asymptomatic hosts*
	High. Several host can remain asymptomatic even for years (high risks in absence of proper surveillance).
1.8.3	*Incubation time*
	On average months but sometimes years.
1.9	**Variability of the agent**
1.9.1	*Species / Types*
	One major species recognised (*M. bovis*), nonetheless, at least another species (*M. caprae*) can induce tuberculosis in bovine.
1.9.2	*Mutations*
	Yes. Mutation can influence virulence and range of sensitive hosts.
1.9.3	*Number of hosts / vectors (host-specificity)*
	High number of hosts, low host-specificity.
1.10	**Knowledge of host-pathogen interaction**
1.10.1	*Status of knowledge of host-pathogen interaction*
	Good. In addition, bovine tuberculosis provides and valuable experimental model for human tuberculosis.
1.11	**Knowledge of immunology**
1.11.1	*Complete / partial / absent knowledge of humoral immunity*
	Almost complete. In addition, immunity against bovine tuberculosis provides and valuable experimental model for human tuberculosis.
1.11.2	*Complete / partial / absent knowledge of cellular immunity*
	Very good. In addition, immunity against bovine tuberculosis provides and valuable experimental model for human tuberculosis.

2	SOCIO-ECONOMIC IMPACT
2.1	**Impact on production within the region**
2.1.1	*Production losses*
	Lombardy is free of the disease, losses only in case of mandatory culling in according to current laws (surveillance ad control plan, DDUO97 01/12/2011)
2.1.2	*Quality losses*
	Minor (possible quality losses in meat of infected animals).
2.1.3	*Menace to survival of livestock industry*
	None (menace only at farm-level).
2.2	**Economic impact of the control plan**
2.2.1	*Voluntary / mandatory control plan*
	In Lombardy is active a mandatory control plan.
2.2.2	*Actual cost of the surveillance*
	Low. In US some Authors estimated the cost of surveillance in 1 to 2 $ per animal every year. In Lombardy no scientific papers are available, however, according to regional specialists the yearly cost for diagnostic is around 0.90 € per animal (2010 costs, labour not included).
2.2.3	*Outbreak within the region (per year)*
	Low to very low. – 11 outbreaks in 2005. – 14 outbreaks in 2006. – 11 outbreaks in 2007. – 2 outbreaks in 2009. – 4 outbreaks in 2010. – 1 outbreak in 2011.
2.2.4	*Adopted control measures and actual costs*
	In case of outbreak mandatory tests and cull of infected animals (2003 US estimations): – $592.80 for the cull of an adult. – $112.00 for the cull of a calf. – $1390.00 for replacement of a milking cow ($1086.00 for a beef bovine). – $0.31 for every kg of milk lost (indirect losses for cull of milking cows and heifers).
2.3	**Potential economic direct impact**
2.3.1	*Production and movement limited / banned*
	Lombardy is officially free of bovine tuberculosis. Production and movement, at farm-level, are subject to limitations and bans in case of outbreak (suspension or revocation of free-status). Namely: – Milk of infected cows cannot be used for human consumption (destruction or for calves consumption within the farm after high-temperature processing). – Milk of uninfected cows can be stocked, according the regulations, after proper high-temperature processing. – Trade of raw milk for direct human consumption is banned (DDUO97 01/12/2011). – Trade of semen and embryos not in compliance with Italian laws is banned (Law 126/63; D.P.R. 505/82, 226/92, 241/92).
2.3.2	*Potential economic cost*
	The economic costs are mostly due to control measures.

2.3.3	*Control strategies available (vaccination and therapy / Test-and-cull / Stamping out)*
	Three control approach may be used, depending on epidemiological status (DDUO97 01/12/2011): – Test and cull. – Modified stamping out (cull of infected and suspected animals). – Stamping out.
2.3.4	*Costs of intervention (surveillance and control)*
	Surveillance – In US the cost of surveillance is estimated in 1 to 2 $ per animal every year (2003). In Lombardy no published data are available, however, according to regional specialists the yearly cost for diagnostic is around 0.90 € per animal (2010 costs, labour not included). Control – In case of outbreak (2003 US estimations): – $592.80 for the cull of an adult. – $112.00 for the cull of a calf. – $1390.00 for replacement of a milking cow ($1086.00 for a beef bovine). – $0.31 for every kg of milk lost (indirect losses for cull of milking cows and heifers). In addition, administrative costs (salary for professionals involved in the control plan), refunds for culled animals, and costs related to equipment used during interventions.
2.4	**Potential economic indirect impact**
2.4.1	*Consequences on the distribution of the products*
	Lombardy is officially free of bovine tuberculosis, no indirect consequences on the distribution of the products (only direct in case of outbreak).
2.4.2	*Market value losses*
	None reported at the moment.
2.4.3	*Nation-wide ban of distribution*
	None (only direct consequences and merely at farm-level).
2.4.4	*Cost of treatments and control of the disease in humans*
	Neglectable at regional-level. Costs for single patient may be very high. However, in Italy, during the last years, only 5 to 10 confirmed human cases per year. Specifically, costs of treatments and control of the disease in humans due to: – Prolonged pharmacological treatments. – Hospitalisation for days to months. – Labour of medical personnel. – Possible (but uncommon) long-lasting disability as consequence of the disease.
2.4.5	*Tourism losses and menace to biodiversity*
	Low but not numerically estimated. Potential losses in tourism and hunting activities in case of high prevalence the disease in the wildlife.
2.4.6	*Restriction on the entire productive system*
	None at the moment, very unlikely in future.
3	**IMPACT ON PUBLIC HEALTH**
3.1	**Relevance in laws (locals to international)**
3.1.1	*Relevance in laws (region / nation / EU / worldwide)*
	International (OIE Terrestrial Animal Health Code – Chapter 11.6).
3.2	**Zoonotic potential**
3.2.1	*Possibility of transmission between animals and humans (yes / no / unclear)*
	Yes.

3.2.2	*Likelihood of transmission between animals and humans*
	Very low in countries where pasteurisation is widespread and eradication plans are mandatory. In EU, during 2008, only 115 confirmed cases (0.02 cases / 100,000 inhabitants). Confirmed cases in Italy during the last years (>0.01 cases / 100,000 inhabitants): – 27 cases from 2004 to 2007. – 1 case in 2008. – 5 cases in 2009.
3.2.3	*Route of transmission from animals to humans (direct, indirect; vector, food, environment, airborne)*
	The most important route of transmission, from animals to humans, is through ingestion of raw infected milk. Airborne transmission and infection through cutaneous wounds are also possible but uncommon.
3.2.4	*Species barrier*
	Very low to none.
3.2.5	*Virulence factors*
	Several virulence factors (molecules of the wall cell or secreted peptides) induce various degrees of virulence in different *M. bovis* strain, among the most important: – Trehalose 6,6'-dimycolate (TDM or Cord Factor), glycolipid of the wall cell, seems to impede leukocyte extravasation and to be toxic for leukocytes and hepatocytes. – Lipoarabinomannan (LAM), glycolipid of the wall cell, provides protection against reactive oxygen species (ROS) and seems to inhibit phagocytosis. – The antigen 85 complex (secretion proteins) seems to impair cellular immunity. – Proteins encoded by *mce* operons, seem to be pivotal for invasion of the epitheliums, and entry and survival inside macrophages. – Stress proteins (superoxide dismutase, alkyl hydroperoxidase) provide protection against ROS.
3.2.6	*Likelihood of underestimate human cases*
	Likely. Infected humans may remain asymptomatic for years.
3.3	**Likelihood of occurrence**
3.3.1	*Probability of occurrence*
	Very low (in Italy >0.01 cases / 100,000 inhabitants per year).
3.4	**Spread in humans**
3.4.1	*Likelihood of transmission between humans*
	Very rare between immune-competent humans. Possible between immunocompromised patients.
3.4.2	*Route of transmission between humans (direct, indirect)*
	Airborne transmission between humans in close contact.
3.5	**Impact on human health**
3.5.1	*Severity of the disease*
	Variable (from asymptomatic to life-threating). In addition, the firsts symptoms may occur years after the contagion. Clinical signs vary based on localization of the lesions (e.g.: lungs, liver, kidney) and severity of the disease.
3.5.2	*Symptoms duration and time off work length*
	The disease is typically chronic. Clinical infections require a prompt treatment (the disease can be fatal) and a long time off work, hospitalization may also be prolonged.
3.5.3	*Permanent damages*
	Possible but uncommon. Permanent damages depend on severity of the diseases and promptness of the treatment.

3.5.4	*Mortality*
	Sporadic. Mortality may occur if the disease is left untreated or in immunocompromised patients.
3.6	**Impact on food safety**
3.6.1	*Likelihood of infection / intoxication due to infected / contaminated food*
	Variable (very unlikely in Lombardy). Foodborne transmission may occur due to ingestion of infected raw milk or other infected foods.
3.6.2	*Infectious / toxic dose*
	Infectious dose is not exactly clear but it seems high (estimated in millions of UFC).
3.6.3	*Mandatory precautions*
	Lombardy is free of bovine tuberculosis. Mandatory precautions are required in case of suspension or loss of the free-status such as (DR 97 01/12/2011): – Milk of infected cows cannot be used for human consumption (destruction or for calves consumption within the farm after high-temperature processing). – Milk of uninfected cows can be stocked, according the regulations, after proper high-temperature processing. – Trade of raw milk for direct human consumption is banned.
3.7	**Bioterrorism potential**
3.7.1	*Potential to cause substantial harm in humans*
	Bioterroristic potential of *M. bovis* is neglectable (not in included in category A or B of CDC list of potential bioterrorism agents).
3.7.2	*Agent availability*
	Not applicable.
3.7.3	*Facility of use and conservation (Labs / trained professionals / sole person)*
	Not applicable.
4	**IMPACT ON TRADE**
4.1	**Impact on regional trade due to current laws**
4.1.1	*Ban / Limitations on trade (positive animal only, herd, limited area, region-wide)*
	Lombardy is free of bovine tuberculosis (no limitations on trade between free farms). Ban and limitations are possible at farm-level in case of suspension or loss of the free-status, in compliance with the current laws (DDUO97 12/01/11).
4.1.2	*List of banned products*
	Lombardy is free of bovine tuberculosis (no banned products). In case of suspension or loss of the free-status, bans are on: – Sell of raw milk for direct human (DDUO97 12/01/11) – Trade of semen and embryos not in compliance with the Italian laws (Law 126/63; D.P.R. 505/82, 226/92, 241/92) and EU laws (88/407/EEC; 2003/43/EC).
4.1.3	*Free-status loss*
	Possible suspension or loss of the free-status; in case of outbreak the zone of control is usually at farm-level (DDUO97 12/01/2011).
4.1.4	*Difficulty and time needed to regain free-status*
	Suspension – medium to low difficulty: suspensions can occur due to the presence of suspected animals within the farm or the violations of laws on animal movements. In order to regain the free stratus intradermoreaction test (IDT) must be negative for all animals older than six weeks within the farm. These animals must be tested at least 42 days after the cull of the suspected animals or the violations (DDUO97 12/01/2011). Loss – medium difficulty: losses of free-status occur in case of outbreak. free-status can be regained four months after the cull of all infected animals if, in the farm, all the animals older than six weeks are negative to IDT (DDUO97 12/01/2011).

4.2	**Impact on national / EU trade due to current laws**
4.2.1	*Ban / Limitations on trade (positive animal only, herd, limited area, nation-wide)*
	Lombardy is free of bovine tuberculosis (no limitations on trade between free farms). Ban and limitations are possible at farm-level in case of suspension or loss of the free-status, in compliance with the current laws (64/432/EEC).
4.2.2	*List of banned products*
	Lombardy is free of bovine tuberculosis (no banned products). Possible bans on trade if semen or embryos are not in compliance with the Italian laws (Law 126/63; D.P.R. 505/82, 226/92, 241/92) and EU laws (88/407/EEC; 2003/43/EC).
4.2.3	*Free-status loss*
	Italy is not officially free of bovine tuberculosis.
4.3	**Impact on international trade due to current laws**
4.3.1	*Ban / Limitations on trade (positive animal only, herd, limited area, nation-wide or EU-wide)*
	Lombardy is free of bovine tuberculosis (no limitations on trade between free farms among the 180 OIE member states). Ban and limitations are possible at farm-level in case of suspension or loss of the free-status, in compliance with the current laws (OIE Terrestrial Animal Health Code – Chapter 11.6).
4.3.2	*List of banned products*
	Lombardy is free of bovine tuberculosis (no banned products). Products subject to possible banned are: milk and meat (in case of suspension or loss of the free-status), semen or embryos (if not in compliance with the current laws).
4.3.3	*Free-status loss*
	Italy is not officially free of bovine tuberculosis.
4.3.5	*Countries with particularly strict laws (about the disease)*
	Not applicable (current laws are already strict).
4.4	**Potential for zoning**
4.4.1	*Zone of control size*
	Zoning is conceivable at farm-level (DDUO97 12/01/2011).
5	**IMPACT ON ANIMAL WELFARE**
5.1	**Potential impact on animal welfare (duration)**
5.1.1	*Presence and duration of animal welfare damages*
	Chronic infections may impair animal welfare, mortality is rare. Severity of the diseases may vary based on virulence of the strain, susceptibility of host species/breed, host immune status and immune response.
5.2	**Potential frequency of severe distress**
5.2.1	*Percentage of animal with severe distress*
	Variable (from 0 to 10% of infected cattle may develop severe distress).
5.3	**Severity / reversibility of the disease**
5.3.1	*Severity of the disease and reversibility of the damages*
	Severity of the disease can vary, severe infections with irreversible damages are possible. Bovine tuberculosis is typically a chronic diseases, however, infections with a relatively rapid progression may occur. Clinical symptoms can vary depending on the localization and the dimension of the lesions induced by *M. bovis*. The most common signs are fever (wavelike), progressive impairment, weakness, and lack of appetite. In addition, severe infections usually induce strong respiratory symptoms and severe distress.
5.3.2	*Available therapies and their effectiveness*
	Not applicable. Treatments are forbidden by laws and infected animals must be culled.

5.4	**Impact on animal freedom**
5.4.1	*Potential restriction on the "Five Freedom" (freedom from fear and distress NOT included)*
	In countries that adopt efficient surveillance control plans infected animals are usually identified before the manifestation of any symptoms. Clinical infections may impede: – Freedom from pain, injury or disease. – Freedom from hunger and thirst (in case of extremely severe infections).
6	**CONTROL TOOLS**
6.1	**Proper tools for diagnosis**
6.1.1	*Validated kits availability within the nation*
	Not applicable (diagnosis regulated by current laws).
6.1.2	*Laws that rule the surveillance*
	Common surveillance on all farms (periodical), all animal movements, and in case of outbreak (DDUO97 12/01/2011): – IDT to all bovine and buffaloes older than six weeks in compliance with the control plan. – Accurate Post-mortem visit in the slaughterhouse and sampling of all the suspicious lesion (the sample will be tested by the zooprophylactic institute competent for the zone). Specific surveillance in case of outbreak (DDUO97 12/01/2011): – Agent isolation. – Interferon-gamma test in possible in some cases (see "*allegato D*" of DDUO97 12/01/2011).
6.1.3	*Techniques described by international organisation (OIE, WHO, UE, etc.)*
	OIE MANUAL OF DIAGNOSTIC TESTS AND VACCINES FOR TERRESTRIAL ANIMALS (Chap. 2.4.7): a) <u>Identification of BTV</u> – *Microscopic examination.* – *Culture* (OIE gold standard). – *Nucleic acid recognition methods* (PCR , spoligotyping, restriction endonuclease analysis (REA), restriction fragment length polymorphism (RFLP), mycobacterial interspersed repetitive units (MIRU)-variable number tandem repeat (VNTR) typing) b) <u>Tuberculin test</u> (delayed hypersensitivity test prescribed test for international trade) c) <u>Blood-based laboratory tests</u> – *Gamma-interferon assay* (alternative test for international trade, stimulation with avian and bovine PPD-tuberculin, ESAT 6, or CFP-10) – *Lymphocyte proliferation assay* (available only in few labs and very expensive, for research purposes only). – *ELISA techniques.*
6.1.4	*DIVA test possibility / obligation of use*
	None available. Gamma-interferon assay based on stimulation with ESAT 6, or CFP-10 may discriminate between infected and vaccinated animals.
6.1.5	*Overall judgement on tools for control*
	The tools for control adopted in Lombardy seem to be adequate to eradicate the disease at farm-level, and thus, maintaining the free-status.
6.2	**Proper tools for prevention (within the region/area)**
6.2.1	*Obstacles / incentives to prevention*
	Incentives – Mandatory plan of surveillance, control, and eradication. Obstacles – Control of disease in the wildlife, illicit introduction of animal from areas at risk, illegal use of antibiotics.

6.2.2	*Available prevention strategies and their efficacy*
	The most important available prevention strategies are: – Strict controls on animal movements. – High standards of biosecurity. – Avoid direct or indirect contacts between sensitive domestic hosts and reservoirs wild species – Proper epidemiological surveillance
6.2.3	*Commercial vaccines availability in EU / Worldwide*
	None, no commercial vaccine available within EU.
6.2.4	*Marker vaccines availability in EU / Worldwide*
	None.
6.2.5	*Vaccination efficacy*
	Counter-productive when a proper plan for surveillance, control, and eradication is active. Moreover, in countries where vaccination is permitted the efficacy of immunisation interventions is inconsistent.
6.2.6	*Laws that rule vaccination*
	Vaccination is forbidden (by the ministerial decree number 592 of December 15 1995).
6.3	**Proper tools for control (within the region/area)**
6.3.1	*Obstacles / Incentives to control*
	Incentives – Mandatory plan of surveillance, control, and eradication with a usually favourable cost-benefit. For example, in US the neat benefit of the plan for 2003 was 159 millions of dollars. In Ireland cost-benefit analysis has (CBA) estimated high benefits (benefit surpass cost for an 85%). Obstacles – Control of disease in the wildlife, illicit introduction of animal from areas where the disease is present, illegal use of antibiotics, risks of unfavourable cost-benefit if the control measures are not optimised (as reported by English and Spanish Authors).
6.3.2	*Available control strategies and their efficacy*
	Various strategies available such as: – Test and segregation (low efficacy). – Test and cull (low efficacy when used alone). – Strict plan for surveillance, control and eradication (good efficacy to gain the free-status). – Surveillance and control of the wildlife (costly).
6.3.3	*Laws that rule control strategy*
	The plan of surveillance, control and eradication is mandatory for every farm, within Lombardy, that breed bovine or buffaloes (DDUO97 12/01/2011).
6.4	**Proper tools for therapy**
6.4.1	*Therapeutic protocol in use (cure and prophylaxis)*
	Treatment is forbidden and detrimental for both the control plan and the public health (risks of antibiotic resistance). Moreover, therapeutic protocol exits but they are expensive and provide inconsistent results.
6.4.2	*Laws that rule therapies*
	Therapies are strictly forbidden, in addition, if suspicious lesions are founded during post-mortem visits the bulk milk of the farm where the animal was from must be tested for isoniazid residual (DDUO97 12/01/2011).
6.4.3	*Residual risks / suspension time*
	Non applicable.

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Appendix B: Bovine Mastitis (*S. aureus*)

SCORECARD [Last update 08-30-2013]								
BOVINE MASTITIS (*S. aureus*)		**SCORE = 547**						
Criteria		**Score**					**Coef**	**Tot**
1	**Relevance Of The Disease**	**1**	**2**	**3**	**4**	**5**	**1.43**	**64.3**
1.1	Presence of the disease				4		22.86	
1.2	Frequency of the disease				4			
1.3	Number of species involved		2				2.86	
1.4	Speed of spread			3			4.29	
1.5	Vectors as reservoir and potential source of the disease		2				2.86	
1.6	Risk of spread to susceptible species				4		5.71	
1.7	Wildlife as reservoir and potential source of the disease		2				2.86	
1.8	Potential for silent spread					5	7.14	
1.9	Variability of the agent					5	7.14	
1.10	Knowledge of host-pathogen interaction			3			4.29	
1.11	Knowledge of immunology			3			4.29	
2	**Socio-Economic Impact**	**1**	**2**	**3**	**4**	**5**	**10.00**	**120**
2.1	Impact on production within the region				4		40.00	
2.2	Economic impact of the control plan		2				20.00	
2.3	Potential economic direct impact				4		40.00	
2.4	Potential economic indirect impact		2				20.00	
3	**Impact On Public Health**	**1**	**2**	**3**	**4**	**5**	**8.57**	**189**
3.1	Relevance in laws (locals to international)				4		34.29	
3.2	Zoonotic potential				4		34.29	
3.3	Likelihood of occurrence			3			25.71	
3.4	Spread in humans		2				17.14	
3.5	Impact on human health			3			25.71	
3.6	Impact on food safety				4		34.29	
3.7	Bioterrorism potential		2				17.14	
4	**Impact On Trade**	**1**	**2**	**3**	**4**	**5**	**10.00**	**60**
4.1	Impact on regional trade due to current laws		2				20.00	
4.2	Impact on national / EU trade due to current laws		2				20.00	
4.3	Impact on international trade due to current laws	1					10.00	
4.4	Potential for zoning	1					10.00	
5	**Impact On Animal Welfare**	**1**	**2**	**3**	**4**	**5**	**2.86**	**54**
5.1	Potential impact on animal welfare (duration)				4		34.29	
5.2	Potential frequency of severe distress			3				
5.3	Severity / reversibility of the disease				4		11.43	
5.4	Impact on animal freedom			3			8.57	
6	**Control Tools**	**1**	**2**	**3**	**4**	**5**	**5.00**	**60**
6.1	Proper tools for diagnosis		2				10.00	
6.2	Proper tools for prevention (within the region/area)				4		20.00	
6.3	Proper tools for control (within the region/area)			3			15.00	
6.4	Proper tools for therapy			3			15.00	

	Summary	Crit.	Coeff.	Weight
1	Relevance Of The Disease	64%	1	100
2	Socio-Economic Impact	60%	2	200
3	Impact On Public Health	63%	3	300
4	Impact On Trade	30%	2	200
5	Impact On Animal Welfare	54%	1	100
6	Control Tools	60%	1	100

	SCORECARD FORM [Last update 08-30-2013]
Extra	**DISEASE INFORMATION**
E.1.1	*Name of the disease*
	Bovine mastitis (*S. aureus*).
E.1.2	*Aetiological agent/s*
	Staphylococcus aureus. Gram-positive coccal bacterium; facultative anaerobic; asporigen; immobile; frequently β-haemolytic; catalase and coagulase positive (uncommon strains may not express coagulase).
E.1.3	*Brief description*
	Bovine mastitis by *S. aureus* is common worldwide and represents one of the main cause of losses in cattle. *S. aureus* is a major agent of contagious mastitis, however, clinical cases were became less common in the last decade. Furthermore, these cases are usually mild or moderate. Subclinical cases represents the foremost source of losses within the herds. Contagious mastitis control is based on some key-points namely correct milking practices; segregation of infectious bovines; rational and aimed dry cow therapy; cull of subject with chronic infection. *S. aureus* can produce heat-stable enterotoxins (SE) that may induce food poisoning in humans. These toxicosis are usually mild and most patients recover after one to three days (in rare cases toxic shock is possible). Besides, *S. aureus* can colonize different species (human being included) without causing disease.
1	**RELEVANCE OF THE DISEASE**
1.1 **1.2**	**Presence and frequency of the disease within the region / outside the region**
1.1.1	*Presence and frequency of the disease within the region*
1.2.1	In Lombardy region epidemiological data suggest a prevalence of *S. aureus* intra-mammary infections of 30% at farm-level and 12 – 15% at animal-level. In farms that sell raw milk for direct human consumption mandatory controls founded 2.9% of the bulk milk samples positive (>100 cfu/ml) for coagulase-positive staphylococci (CPS) in 2007 and 8.6% of samples in 2008.
1.1.2	*Presence and frequency of the disease within near region / country*
1.2.2	*S. aureus* mastitis represent a worldwide problem. In EU a German study founded a prevalence of 21.8%; in Finland 10.1%; in Belgium 19%; in Netherlands 5 – 15% and in a Norway research over 60% of samples were found positive.
1.1.3	*Frequency of epidemics (specify where)*
1.2.3	Agent is widespread worldwide with different prevalence, without real epidemics in large areas. However, epidemics and endemics at farm-level are possible.

1.1.4	*Animal Hosts / Vectors / Environment as source of disease*
1.2.4	Some strains are particularly adapted to invade mammary gland. In these strains the main source of contagion are infected quarters. Additionally, wounded teat skin and heifers (as reservoir or fomite) can be an important source of spread. *S. aureus* can colonize and invade a large number of animal species (human included). However, different species (other than cow) do not seem to have a relevant role in transmission of strains better adapted to infect the bovine mammary gland. These species may sporadically transmit other, less adapted, strains. Infection can be spread by flies as mechanical vector, though, the epidemiological importance of these arthropods is not clear yet (likely a minor role). Milking equipment represents the only relevant environmental source of disease.
1.1.5	*Seasonal cycles / outbreak influenced by climate anomalies*
1.2.5	Seasonal cycles do not seem to be influent for spread. Climatic anomalies, such as exceptional cold, may impair the natural barrier of teats and mammary gland and favour infections.
1.1.6	*Factors that facilitate the presence of the agent (scarce hygiene, biosafety, management, etc.)*
1.2.6	Overall: poor hygiene; inadequate management; animal movements (purchase of infected cows/heifers). Milking: poor milking hygiene; inadequate teats cup linear; poor milking practices.
1.1.7	*Stability of the agent within the environment*
1.2.7	High. *S. aureus* may survive for weeks in suitable environment. However, with exception of milking equipment, stability of the agent within the environment do not play an important role in spread.
1.1.8	*Likelihood of eradication*
1.2.8	None. *S. aureus* can colonize a large number of species without causing disease. Eradication of strains better adapted to infect the bovine mammary gland is possible at farm-level.
1.3	**Number of species involved**
1.3.1	*Number of species involved (specify which ones)*
	Bovine. *S. aureus* can be isolated from a huge number of domestic species such as water buffalo; sheep; goat; pig; equine; poultry; camelids; rodents; dog and cats. Furthermore, *S. aureus* represent a major cause of mastitis in sheep and goats. However, strains from these species seem to be genetically different from strains particularly adapted to invade mammary gland and they are an uncommon cause of mastitis in cattle.
1.4	**Speed of spread**
1.4.1	*Speed of spread within the farm*
	Variable. Relatively fast in absence of control and adequate hygiene and management practices.
1.4.2	*Speed of spread between farms*
	Variable. It depends on infected animal movements without control.
1.4.3	*Likelihood of spread without hosts movement outside the farm*
	Unlikely / none.
1.5	**Vectors as *reservoir* and potential source of the disease**
1.5.1	*Disease cycle influenced by vectors*
	Flies can act as mechanical vector. The role of these arthropods is unclear, flies seem to be relevant in transmission only in areas where they can reproduce in large numbers without control.
1.5.2	*Presence of the vectors within the regional / national territory*
	Not applicable (usually neglectable for eradication at farm-level).

1.5.3	*Presence of the vectors influenced by peculiar areas / climates*
	Not applicable (usually neglectable for eradication at farm-level).
1.5.4	*Vectors likelihood of survival, reproduction, spread the disease, act as reservoir*
	Not applicable (usually neglectable for eradication at farm-level).
1.6	**Risk of spread to susceptible species**
1.6.1	*Likelihood of transmission*
	Medium to high during milking without control of risk factors. Unlikely outside milking (for contagious mastitis).
1.6.2	*Route of transmission*
	Mostly through indirect contact among cows during milking; transmission due to direct contact between mammary gland of different animals is unlikely. Heifers can act as reservoir or fomite. Other routes of transmission (from infection in different organs, from other species, through vectors) are sporadic and usually neglectable for eradication purposes at farm-level.
1.6.3	*Peculiar condition that influence transmission*
	Poor hygiene; inadequate management; low biosecurity level (infected cows/heifers); inadequate milking practices.
1.7	**Wildlife as reservoir and potential source of the disease**
1.7.1	*Species involved*
	High number of species can be colonized or infected. However, strains from these species seem to be genetically different from strains particularly adapted to invade mammary gland and wildlife seems to be an unlikely source of infection.
1.7.2	*wildlife / livestock and pet / human interactions*
	Not applicable.
1.7.3	*Endangered species involved*
	Not applicable.
1.8	**Potential for silent spread**
1.8.1	*Likelihood of recognition due to the clinical symptoms*
	Very low. Clinical mastitis by *S. aureus* are uncommon in Lombardy (majority of cases remain subclinical) and the symptoms are not dissimilar to other clinical mastitis.
1.8.2	*Spread by subclinical / asymptomatic hosts*
	High. Subclinical cases represent the majority of *S. aureus* mastitis and laboratory diagnosis (SCC and bacteriology) is mandatory. Furthermore, diagnosis can be difficult due to the low or intermittent shedding rates of *S. aureus.*
1.8.3	*Incubation time*
	Variable (days to weeks). Clinical manifestations, when present, are dependent on single animal conditions, strain virulence and environment (stress).
1.9	**Variability of the agent**
1.9.1	*Species / Types*
	A large number of strains are known, these strains can colonize or infect a wide range of hosts and they can occupy various niches. Some strains are particularly adapted to invade mammary gland and have peculiar genetic characteristics.
1.9.2	*Mutations*
	Common. Various studies has enlightened the genetic differences among strains that occupy diverse niches. Mutations that lead to SCV (small-colony variant) and MRSA (Methicillin-resistant Staphylococcus aureus) are matter of serious concern.

1.9.3	*Number of hosts / vectors (host-specificity)*
	Variable host-specificity. *S. aureus* can colonize a large number of hosts and vectors. Nevertheless, some strains seems to adapted to a specific "*niche*" (e.g. mammary glands) and they may rarely isolated outside their niche. Genetic characteristics of these strains and how they interact with different hosts need further investigations. The relevance of "genetic information" shift between human and bovine strains are not fully known; this shift need to be further clarified due to the growing antibiotic resistances and isolation of MRSA.
1.10	**Knowledge of host-pathogen interaction**
1.10.1	*Status of knowledge of host-pathogen interaction*
	Partial. *S. aureus* ability to colonize bovine mammary gland; to invade the epithelium and to localize within the interstices are matters of particular interest due to the negative consequences on therapy. Besides, *S. aureus* can infect the mammary gland without visible symptoms but with an important increase in SCC and cause relevant economic losses. Some genetic characteristics and how they affect host-pathogen interaction are still unclear. The role of heifers in spread needs further investigations. Host-specificity, host shift and adaptation capabilities of different strains are only partially known. Some virulence factors remain unidentified; in order to improve Status of knowledge of host-pathogen interaction and possible vaccines a better knowledge of these virulence factor and their regulation are required.
1.11	**Knowledge of immunology**
1.11.1	*Complete / partial / absent knowledge of humoral immunity*
	Partial. Humoral immunity does not seem to have relevant in protection against mammary gland infection and it poses a great limitation in killed vaccine development. Roles of neutralising antibody and humoral-cellular immunity interactions in mammary gland protection need further investigation.
1.11.2	*Complete / partial / absent knowledge of cellular immunity*
	Partial. Role of cellular immunity in pathogenesis and protection needs further investigations.
2	**SOCIO-ECONOMIC IMPACT**
2.1	**Impact on production within the region**
2.1.1	*Production losses*
	Mastitis represents one of the major causes of economic losses in cattle. In Lombardy, production drop due to the disease determines losses for 2 million of euros per year. Costs of subclinical mastitis are 60 – 350€ per cattle; in clinical cases 136 – 267€ represent the expenses for therapy. Direct losses: – Drop of milk production – Reduction of milk quality – Infected milk waste or due to suspension time (therapy) – Cull of cows with chronic infection / refractory to therapy + replacement expenses – Drop of cheese yield – Mortality (uncommon in *S. aureus* mastitis) Indirect losses: – Routine expenses (e.g. feed) for cows with less milk yield or with waste milk – Drop of reproductive performances – Loss of future income due to cull / drying-off mammary quarters infected with staphylococcus aureus Furthermore, outcomes for veterinary interventions (visit, diagnosis and therapy); costs linked to control plans (if present) and ban of product (e.g. raw milk).

2.1.2	*Quality losses*
	Relevant quality losses of milk: – Increase in somatic cells count (drop in milk price and interference with cheese production) – Drop in lactose yield (5-20%) – Drop in casein yield (6-18%) – Drop in fat yield (5-12%) – Reduction of mineral salt (Ca, P e K) Furthermore, plasminogen concentration in milk can increase due to mammary gland infections, rennet activates plasminogen in plasmin during cheese production and plasmin reduces concentration of k-casein, which may result in a delayed curd formation. The increase of γ-globulin in milk, during intramammary infections, impairs thermic stability of milk and may alter cheese production processes.
2.1.3	*Menace to survival of livestock industry*
	None. Economic impact is significant; however, the disease does not menace survival of livestock industry (only sole farms).
2.2	**Economic impact of the control plan**
2.2.1	*Voluntary / mandatory control plan*
	No official plan available in Lombardy (neither mandatory nor voluntary). Some farmers implemented a plan of eradication on a voluntary basis.
2.2.2	*Actual cost of the surveillance*
	Not applicable.
2.2.3	*Outbreak within the region (per year)*
	Not applicable.
2.2.4	*Adopted control measures and actual costs*
	Not applicable.
2.3	**Potential economic direct impact**
2.3.1	*Production and movement limited / banned*
	Suspension or ban if raw milk for direct human consumption does not comply with the law (Circ. 19/SAN/07).
2.3.2	*Potential economic cost*
	Losses in milk yield can be significant, namely, 150 – 300 litres per cows. Furthermore, infected cows may undergo delay in first heat; fertility drop; higher risk of foetal death. Besides, cows with a previous clinical mastitis can present reduction in reproductive performances (excessive days open).
2.3.3	*Control strategies available (vaccination and therapy / Test-and-cull / Stamping out)*
	Several strategies are available: – High standards in biosecurity, hygiene and management (pivotal pre-requisite for control, the costs may vary). – Dry and/or Milking therapy (ineffective alone at farm-level and inconsistent results even at animal-level) – Vaccination (poor efficacy for bovine mastitis by *S. aureus*). – Eradication plan based only on “test-and-cull” (unsustainable with high prevalence). – Eradication plan based on segregation (effective when applicable).

2.3.4	*Costs of intervention (surveillance and control)*
	Low for screening (bulk milk). In a rational and effective eradication plan cost of surveillance should be around 100€ per cows every year (average duration 2 years). Expenses of control may vary. Outcomes for culling in a farm with high prevalence are huge. Costs of therapy during milk are commonly 136 – 267€ per clinical case; expenses of ration dry cow therapy should be about 10€ per cow. Costs of an adequate control plan can be high, they depends on prevalence of infection and status of farm (biosecurity, hygiene and management). However, the costs/benefit ratio is favourable on the long-run.
2.4	**Potential economic indirect impact**
2.4.1	*Consequences on the distribution of the products*
	Mild / None. Consequences only on the distribution of raw milk for direct human consumption.
2.4.2	*Market value losses*
	Neglectable (at the moment). Potential market value losses due to fear of the disease caused by media is unlikely. Nevertheless, if severe *S. aureus* infections in humans (i.e. hospital-acquired) will gain more exposure, potential losses in milk sales will be possible even if intramammary infections by *S. aureus* in cows are totally unrelated. Furthermore, negative economic indirect losses will be conceivable if bovine *S. aureus* will show a more relevant role in MRSA epidemiology and/or as a source of antibiotic-resistant strains for humans.
2.4.3	*Nation-wide ban of distribution*
	None.
2.4.4	*Cost of treatments and control of the disease in humans*
	Low. Food poising due to ingestion of SE is usually not severe and self-heal is frequent, hospitalization is rarely required. Hospital-acquired infections caused by *S. aureus* can be severe (sepsis and/or encephalitis) and may result in death, hospitalization is required and the cost of treatments is usually high. However, these infections and bovine mastitis by *S. aureus* are not related.
2.4.5	*Tourism losses and menace to biodiversity*
	Never described.
2.4.6	*Restriction on the entire productive system*
	None.
3	**IMPACT ON PUBLIC HEALTH**
3.1	**Relevance in laws (locals to international)**
3.1.1	*Relevance in laws (region / nation / EU / worldwide)*
	EU-wide.
3.2	**Zoonotic potential**
3.2.1	*Possibility of transmission between animals and humans (yes / no / unclear)*
	Low. Bovine strains rarely infect humans; however, food poisoning due to *S. aureus* toxins is likely to be common.
3.2.2	*Likelihood of transmission between animals and humans*
	Low but exact frequency is unknown. Food poisoning due to SE is not uncommon, nonetheless, epidemiology estimations are difficult because other coagulase-positive staphylococci (CNS), like *S. intermedius* and *S. hyicus* (although sometimes they do not express coagulase), may produce SE and lead to misdiagnosis. CNS do not seem to play a relevant role in SE food poisoning.

3.2.3	*Route of transmission from animals to humans (direct, indirect; vector, food, environment, airborne)*
	Infections: Direct contact with infected cows. Poisoning: Ingestion of contaminated food or direct contact if mucosae is accidentally exposed to Staphylococcal enterotoxin B (SEB).
3.2.4	*Species barrier*
	Medium barriers for infections (different strains in diverse species). None for food poisoning.
3.2.5	*Virulence factors*
	Plenty virulence factors are known, others are still putative. Additionally, expression and regulation of virulence factors need further investigations. Virulence of different strains is correlated to their ability of produce toxins and certain cell-wall molecules. The most important virulence factors are: – *Enterotoxins* (*SEs*), large number of superantigens, SEB is one of the most dangerous for humans. – *Superantigen-like proteins* (SSLs), host immune evasion/modulation; molecular structure similar to SEs without superantigen activity and no/unknown gastro-enteric toxicity. – *Exfoliative toxins* (EF*A* and EF*B*), proteases pivotal for cutaneous infections. – *Leukocidin PV* (*Panton-Valentine*), pore-forming toxin, one of the key factors for necrotizing pneumonia. Common in MRSA strains. – *Protein A*, binds IgG and inhibits phagocytosis. – *Penicillin-binding protein 2a* (*PB2a*), encoded by *mecA* gene, provides β-Lactam antibiotic résistance. – *Fibronectin-binding protein*, facilitates host invasion. – *Coagulase*, facilitates host invasion, important for bacteriological and molecular diagnosis. – *Capsular polysaccharides*, permits phagocytosis resistance, host adhesion and biofilm formation. – Other toxins (*alpha-toxin, beta-haemolysins, gamma--haemolysins, delta-haemolysins*). – Other enzymes (*proteases A-F, endopeptidase V8, hyaluronidase, staphylokinase*). – *Biofilm*, (complex agglomerate of bacteria cells, organic matrix and water), adhesion to various surfaces and protective environment against host defences and antibiotics.
3.2.6	*Likelihood of underestimate human cases*
	Food poisoning by *S. aureus* toxins is likely to be underestimated due to its evolution. in particular, these poisonings usually present mild to medium non-specific symptoms and quick self-heal is common.
3.3	**Likelihood of occurrence**
3.3.1	*Probability of occurrence*
	Very low for infections, relatively high for food poisonings. For example, in 1993-'98 WHO reported 574 outbreaks in different European states (French, Germany, Switzerland, Spain, Portugal and countries of Scandinavia); Spain and French suffered the larger number of outbreaks.
3.4	**Spread in humans**
3.4.1	*Likelihood of transmission between humans*
	Unlikely for strains particularly adapted to invade mammary gland (possible for human strains). None for food poisonings.
3.4.2	*Route of transmission between humans (direct, indirect)*
	Not applicable (direct and indirect for human strains).

3.5	**Impact on human health**
3.5.1	*Severity of the disease*
	Mild to medium for food poison. Symptoms like nausea, abdominal pain, vomit and diarrhoea appear 1 - 6 hours after ingestion. SEB accidental inhalation causes both respiratory (cough, dyspnoea, thoracic pain) and general (fever, muscular pain, cephalea) symptoms. Hospital-acquired infections may be severe and lead to shock, neurological damages and deaths. However, these infection are unrelated to bovine mastitis.
3.5.2	*Symptoms duration and time off work length*
	Commonly 1 - 2 days for food poisoning. Toxic shock usually requires long hospitalization; however, is rare.
3.5.3	*Permanent damages*
	Commonly none for food poison. Accidental inhalation of high dose of SEB may causes permanent damages (organs with filter functions and brain).
3.5.4	*Mortality*
	Very rare for food poison (common in hospital-acquired infections).
3.6	**Impact on food safety**
3.6.1	*Likelihood of infection / intoxication due to infected / contaminated food*
	Neglectable for infections but high for intoxication of SEs. Potential sources of contamination are contaminated milk, dairy products (heath-stable toxins) and cross-contaminated foods that allow *S. aureus* growth. SEs are hydro-soluble proteins with superantigen activity; precisely, SEs can bypass normal ways of antigen presentation, directly stimulate T-helper lymphocytes and lead to a massive production of cytokines. Moreover, SEB act as a strong pyrogenic agent in humans, especially when inhaled. Gastro-enteric symptoms are prevalent due to ingestion of SEs while respiratory signs are predominant if inhaled.
3.6.2	*Infectious / toxic dose*
	Low. 10 – 50 ppm in food are enough to cause symptoms. Toxins production is linked to various factors such as strain; temperatures (growth range 10 – 45°C, optimum 37 – 40°C); pH (5.15 – 9.0) and nutrients available.
3.6.3	*Mandatory precautions*
	Raw milk for direct human consumption must have less than 100 cfu/ml of CPS (19/SAN/07). For food safety sake it's important to remind that some SEs are heat-stable and may remain active even after a 30 minute boiling, they are resistant to main proteases (trypsin, chymotrypsin, rennin and papain) and may resist radiation too.
3.7	**Bioterrorism potential**
3.7.1	*Potential to cause substantial harm in humans*
	Neglectable. Stains adapted to mammary glands infections are genetically different to humans strains and they are an unlikely source of infection. SEs are usually responsible of mild food poisoning. SEB represent the only exception and it may have a potential application in bioterrorism, it is included in category B of Bioterrorism Agents/Diseases List by CDC.
3.7.2	*Agent availability*
	Neglectable for stains that cause bovine mastitis.
3.7.3	*Facility of use and conservation (Labs / trained professionals / sole person)*
	Neglectable for stains that cause bovine mastitis. SEB production and purification require specific expertise and adequate equipment. SEB is stable in environment and easy to store however it's application in a large scale bio-warfare should be limited due to the production limits. It may be used for a small attack in order to receive a great deal of media attention.

4	**IMPACT ON TRADE**
4.1	**Impact on regional trade due to current laws**
4.1.1	*Ban / Limitations on trade (positive animal only, herd, limited area, region-wide)*
	None for animal trade.
4.1.2	*List of banned products*
	Suspension or ban if raw milk for direct human consumption does not comply with the law (Circ. 19/SAN/07).
4.1.3	*Free-status loss*
	Suspension / revocation of license to sell raw milk for direct human consumption.
4.1.4	*Difficulty and time needed to regain free-status*
	Variable, dependent on how quick the milk comply again with the law.
4.2	**Impact on national / EU trade due to current laws**
4.2.1	*Ban / Limitations on trade (positive animal only, herd, limited area, nation-wide)*
	None.
4.2.2	*List of banned products*
	Products that do not comply with the law (EC 2073/2005).
4.2.3	*Free-status loss*
	Not applicable (Italy has not free-status).
4.3	**Impact on international trade due to current laws**
4.3.1	*Ban / Limitations on trade (positive animal only, herd, limited area, nation-wide or EU-wide)*
	None.
4.3.2	*List of banned products*
	Products that do not comply with the law (EC 2073/2005).
4.3.3	*Free-status loss*
	Not applicable (Italy has not free-status).
4.3.5	*Countries with particularly strict laws (about the disease)*
	None.
4.4	**Potential for zoning**
4.4.1	*Zone of control size*
	Not applicable (no official control plan in Lombardy).
5	**IMPACT ON ANIMAL WELFARE**
5.1	**Potential impact on animal welfare (duration)**
5.1.1	*Presence and duration of animal welfare damages*
	Usually mild/medium clinical mastitis with symptoms for few days. These mastitis may evolve in chronic forms that are commonly subclinical.
5.2	**Potential frequency of severe distress**
5.2.1	*Percentage of animal with severe distress*
	Variable (usually none). In Lombardy, clinical mastitis by *S. aureus* are far less frequent than subclinical ones. Furthermore, severe clinical cases are really uncommon. Infected cows with distress, particularly during milking, may pass unnoticed.
5.3	**Severity / reversibility of the disease**
5.3.1	*Severity of the disease and reversibility of the damages*
	Variable. Severe clinical cases are unusual; however, selfheal is rare too. Evolution from both clinical and subclinical infections to chronic mastitis is possible; chronic infected cows may be subject to irreversible damages at mammary gland such as fibrosis, abscesses and obstruction of teats sphincter.

5.3.2	*Available therapies and their effectiveness*
	Variable. Antibiotics during milking may be ineffective (depends on quickness). Accurate dry cow therapy represents the most effective treatment. Peracute mastitis by *S. aureus* are rare; nonetheless, they usually lead to death or cull even with proper treatment (antibiotic, FANS and drip feed).
5.4	**Impact on animal freedom**
5.4.1	*Potential restriction on the "Five Freedom" (freedom from fear and distress NOT included)*
	Restriction on Freedoms: – From pain, injury or disease – To express normal behaviour (fertility problems).
6	**CONTROL TOOLS**
6.1	**Proper tools for diagnosis**
6.1.1	*Validated kits availability within the nation*
	Not applicable.
6.1.2	*Laws that rule the surveillance*
	Mandatory surveillance for farms that sell raw milk for direct human consumption: – Bacteriological tests (CPS must be < 100 UFC/ml) Sampling and test are performed by IZS della Lombardia e dell'Emilia Romagna, as stated in the related law (circ. 19/SAN/07). Regulation (EC) No 2073/2005 on microbiological criteria for foodstuffs establishes: – CPS in Milk and milk powder, n = 5, c = 2, m = 10 cfu/g and M = 100 cfu/g – Absence of staphylococcal enterotoxins in cheese and milk powder (mandatory control if CPS > 10^5 cfu/g), n = 5 (25g each sample)
6.1.3	*Techniques described by international organisation (OIE, WHO, UE, etc.)*
	Regulation (EC) No 2073/2005: – ELISA for enterotoxins (reference EU lab) – BACTEORIOLOGICAL EXAM for CPS following ISO 6888-1 (agar *Baird-Parker*) or ISO 6888-2 (agar RPF) Standards.
6.1.4	*DIVA test possibility / obligation of use*
	None.
6.1.5	*Overall judgement on tools for control*
	Control and eradication are possible at farm-level; a mandatory eradication plan for a large geographical area should be carefully evaluated (epidemiological status, costs/benefits and organizational problems). High standards in biosecurity, management and hygiene are required even before the implementation of a control plan. Particular care should be taken in milking practices and cows turn-over (heifers and purchased animals). Where eradication is not possible farms remain exposed to introduction of new strains and the risk of peak of new infections due to climate anomalies (exceptional cold may impair the natural barriers of teats and mammary gland). An eradication plan based on segregation of infected cows and cull of chronic infected bovines (unresponsive to treatment) presents a favourable cost/benefits ratio on a long period. However, previous mentioned pre-conditions must be satisfied for a successful plan, besides, a reliable protocol in both diagnostic (samples, identification of infected/uninfected, surveillance) and therapy (targeted dry cow therapy and antibiotics during milking) are necessary. Control plans based only on culling may provide varying results and they are less effective than segregation strategies. Vaccination, where available, does not seem to be trustworthy for prevention of new infections.

6.2	**Proper tools for prevention (within the region/area)**
6.2.1	*Obstacles / incentives to prevention*
	Obstacles – Relevant investments at the beginning of program (economic and management); farmer sustains all the expenses for control plan; required high level of coordination and collaboration among farmer, veterinary and laboratory; unavailability of a reliable and cheap on-farm test; vaccination largely ineffective; technical difficulties in management of groups for segregation; very difficult with automatic milking systems. Incentives – Advantageous income/outcome with a proper control plan; required for eradication; mandatory in farms that sell raw milk for direct human consumption.
6.2.2	*Available prevention strategies and their efficacy*
	High standards in biosecurity, management and hygiene are effective in control and prevention (spread within the herd and from purchased cows/heifers). Segregation of infected cows and milking groups based on health status can be effective in prevention and control only if properly managed. Vaccination provides inconsistent results and it is usually ineffective for control and prevention.
6.2.3	*Commercial vaccines availability in EU / Worldwide*
	Only two killed vaccine are available for bovine mastitis by *S. aureus*: – *StarVac*® (Hipra), available in EU. – *Lysigin*® (Boehringer Ingelheim), a lysed culture of polyvalent somatic antigen available in North America.
6.2.4	*Marker vaccines availability in EU / Worldwide*
	None.
6.2.5	*Vaccination efficacy*
	Essentially ineffective. Commercial vaccines are composed of the whole killed bacterium. Killed vaccines stimulate almost only humoral immunity (already present even in healthy cows) that seems to be widely ineffective versus *S. aureus* mastitis. Furthermore, milk may interfere with antibodies activity, some virulence factors are still unknown (possible future targets of vaccination), relevant variances among strains in different farm and geographical areas, and thus, effective vaccines are difficult to develop (adjuvants included). Field studies in several countries observed inconsistent results, in some cases the vaccines were effective in other not. New types of vaccine are under study, with different approach. For example, DNA vaccine seems to be promising because of their capability to induce a cellular immunity without stress.
6.2.6	*Laws that rule vaccination*
	Not applicable (no official control plan in Lombardy).
6.3	**Proper tools for control (within the region/area)**
6.3.1	*Obstacles / Incentives to control*
	Obstacles – Relevant investments at the beginning of program (economic and management); farmer sustains all the expenses for control plan; required high level of coordination and collaboration among farmer, veterinary and laboratory; unavailability of a reliable and cheap on-farm test; vaccination largely ineffective; technical difficulties in management of groups for segregation; very difficult with automatic milking systems. Incentives – Advantageous income/outcome with a proper control plan; mandatory in farms that sell raw milk for direct human consumption.

6.3.2	*Available control strategies and their efficacy*
	Hygiene, biosecurity and appropriate management are pivotal in control plans. Cull of infected cows, as sole control strategy, is usually unsuccessful. A control/eradication plan based on segregation is commonly successful in farms where it can find a proper application. Dry cow therapy is essential in an effective control plan while therapy during milk should be carefully evaluated. Vaccination seems to be ineffective.
6.3.3	*Laws that rule control strategy*
	Mandatory surveillance for farms that sell raw milk for direct human consumption (circ. 19/SAN/07).
6.4	**Proper tools for therapy**
6.4.1	*Therapeutic protocol in use (cure and prophylaxis)*
	Antibiotics for milking cows and, when needed, FANS and drip feed. Dry cow therapy with proper antibiotics (more successful *in S. aureus* mastitis).
6.4.2	*Laws that rule therapies*
	Suspension time required after therapy (length depending on drugs used).
6.4.3	*Residual risks / suspension time*
	Residual risk in milk if suspension time is not followed (usually 2 – 4 days) or with antibiotics misuses (administration of antibiotics for dry period during milking). Residual risk in meat if in culled cows (refractory to therapy) suspension time is not followed.

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Appendix C: Bluetongue

SCORECARD [last update 12-15-2012]									
BLUETONGUE		**SCORE = 499**							
Criteria		**Score**					**Coef**	**Total**
1	**Relevance Of The Disease**	**1**	**2**	**3**	**4**	**5**	**1.43**	**52.9**
1.1	Presence of the disease			3			4.29	
1.2	Frequency of the disease	1						
1.3	Number of species involved				4		5.71	
1.4	Speed of spread					5	7.14	
1.5	Vectors as reservoir and potential source of the disease					5	7.14	
1.6	Risk of spread to susceptible species					5	7.14	
1.7	Wildlife as reservoir and potential source of the disease				4		5.71	
1.8	Potential for silent spread			3			4.29	
1.9	Variability of the agent				4		5.71	
1.10	Knowledge of host-pathogen interaction		2				2.86	
1.11	Knowledge of immunology		2				2.86	
2	**Socio-Economic Impact**	**1**	**2**	**3**	**4**	**5**	**10.00**	**120**
2.1	Impact on production within the region	1					10.00	
2.2	Economic impact of the control plan		2				20.00	
2.3	Potential economic direct impact					5	50.00	
2.4	Potential economic indirect impact				4		40.00	
3	**Impact On Public Health**	**1**	**2**	**3**	**4**	**5**	**8.57**	**60**
3.1	Relevance in laws (locals to international)	1					8.57	
3.2	Zoonotic potential	1					8.57	
3.3	Likelihood of occurrence	1					8.57	
3.4	Spread in humans	1					8.57	
3.5	Impact on human health	1					8.57	
3.6	Impact on food safety	1					8.57	
3.7	Bioterrorism potential	1					8.57	
4	**Impact On Trade**	**1**	**2**	**3**	**4**	**5**	**10**	**130**
4.1	Impact on regional trade due to current laws			3			30	
4.2	Impact on national / EU trade due to current laws			3			30	
4.3	Impact on international trade due to current laws			3			30	
4.4	Potential for zoning				4		40	
5	**Impact On Animal Welfare**	**1**	**2**	**3**	**4**	**5**	**2.86**	**71**
5.1	Potential impact on animal welfare (duration)			3			42.86	
5.2	Potential frequency of severe distress					5		
5.3	Severity / reversibility of the disease					5	14.29	
5.4	Impact on animal freedom					5	14.29	
6	**Control Tools**	**1**	**2**	**3**	**4**	**5**	**5**	**65**
6.1	Proper tools for diagnosis		2				10	
6.2	Proper tools for prevention (within the region/area)				4		20	
6.3	Proper tools for control (within the region/area)			3			15	
6.4	Proper tools for therapy				4		20	

	Summary	Crit.	Coeff	Weight
1	Relevance Of The Disease	53%	1	100
2	Socio-Economic Impact	60%	2	200
3	Impact On Public Health	20%	3	300
4	Impact On Trade	65%	2	200
5	Impact On Animal Welfare	71%	1	100
6	Control Tools	65%	1	100

	SCORECARD FORM [last update 12-15-2012]
Extra	**Disease**
E.1.1	*Name of the disease*
	Bluetongue (*ovine catarrhal fever*)
E.1.2	*Aetiological agent/s*
	Bluetongue virus (BTV), a double-stranded RNA virus without envelope, member of the genus *Orbivirus*, family *Reoviridae*. At the moment there are 24 known BTV serotypes. Genus *Orbivirus* comprises 20 different but related virus. BTV shows close correlation to the serogroup of virus that cause epizootic haemorrhagic disease (EHD) in deer.
E.1.3	*Brief description*
	BTV may infect several ruminants such as sheep, goats, cattle, water buffaloes, and various species of deer and antelopes; although rare, BTV may also infect other species. Biting midges of the genus *Culicoides* act as biological vectors and are the main responsible of BTV spread, indeed, the occurrence of bluetongue in a geographical area is always associated with the presence of these vectors. Other route of transmission are possible (needles, surgical equipment, bull semen) but very unlikely. In the majority of affected species BTV infections are asymptomatic; however, in sheep, deer and antelopes the diseases can be severe with serious distress and high mortality rates (up to 90%). Goats and cattle (BTV-8 infections only) may develop clinical forms but symptoms are usually mild and mortality is rare. Moreover, cattle are the most important amplifying host because of their prolonged viraemia (up to 3 months). Symptoms of bluetongue in sensitive species are often severe and they may include fever, depression, anorexia, excessive salivation, oedema, dyspnoea, nasal discharges, affected mucosae may be hyperaemic, cyanotic and/or ulcerated. Other gastro-intestinal or respiratory symptoms may also occur. Diagnosis is usually based on serological tests and isolation of the virus. The main control strategies are vaccination, biosecurity, control of midges and animal transfers. Bluetongue is not a zoonosis and BTV do not represent a significant threat for human health; nevertheless, one case of infection has been reported in a laboratory technician and a minimum level of security should be adopted while working with BTV.
1	**RELEVANCE OF THE DISEASE**
1.1 **1.2**	**Presence and frequency of the disease within the region / outside the region**
1.1.1	*Presence and frequency of the disease within the region*
1.2.1	None. All the province within Lombardy are declared free for the season (12.19.2010 to 02.23.2011) by the Health Ministry. In Lombardy are active both a serological surveillance plan (farm with sentinel cattle and slaughterhouse) and an entomological surveillance plan (fixed and mobile traps). In Lombardy, at the moment, the mandatory vaccination plan is not active. The last mandatory vaccination campaign was in Mantova province during 2009 due to an outbreak of BTV-8 in Veneto region ("*decreto direzione generale sanità n. 7209 del 02/07/2008*").

1.1.2	*Presence and frequency of the disease within near region / country*
1.2.2	BTV-1, 2, 4, 8, 9 and 16 are the serotypes present in Italy, with different distributions among Italian regions. During the last few years several outbreaks occurred in Italy: – 2002: 392 outbreaks (Sardinia, Molise, Puglia, Basilicata, Calabria, Sicily, Lazio, Campania) – 2003: 3,730 outbreaks (one in Sicily the others in Sardinia). – 2004: 131 outbreaks (Sardinia). – 2006: 238 outbreaks (one in Sicily the others in in Sardinia). – 2007: 19 outbreaks (Veneto e Sardinia). – 2008: 61 outbreaks (Veneto, Piedmont, Sardinia, Sicily). – 2009: 20 outbreaks (Veneto, Sardinia, Sicily, Campania). – 2010: 36 outbreaks (Sardinia, Sicily, Campania). – 2011: 446 outbreaks (440 in Sardinia the others in Sicily and Campania). During the last few years outbreaks occurred in all the neighbour Countries.
1.1.3	*Frequency of epidemics (specify where)*
1.2.3	Within EU outbreaks were uncommon and occurred only in Mediterranean Countries until the end of the nineties. Since 1998 outbreaks and even epidemics became more frequent in Mediterranean area, finally, in 2006 the first epidemic occurred in Northerner European Countries. Precisely: – 1998: an epidemic of bluetongue started in Greece and spread within Mediterranean area. – 2000: BTV-2 in Sicily, Sardinia, and Calabria (also BTV-9). In Sardinia, over 260,000 sheep were culled or died during this epidemic. – 2001: large epidemic in Italy (ended in April 2002), over 250,000 sheep were culled or died during this epidemic. – 2003: BTV-4 in Sardinia (3,729 outbreaks). – 2006: BTV-8 epidemic (over 2,000 outbreaks) started in Netherlands then spread in Germany, Belgium, Luxembourg, and France (bovines were affected too). – 2007: BTV-8 in North European Countries with over 40,000 outbreaks. – 2008: BTV-8 in France with about 38,000 outbreaks.
1.1.4	*Animal Hosts / Vectors / Environment as source of disease*
1.2.4	Affected hosts are susceptible to the disease with different degree, depending on species and breed. Cattle may play a central role (amplifying hosts) in spread of bluetongue due to their prolonged viraemia generally without symptoms. The epidemiological role of *Artiodactyla* (other than ruminants) is still unclear. The presence of bluetongue in a geographical area is possible only if its biological vector is present, particularity female biting midges of various species of *Culicoides.* Although BTV may be persist in a favourable environment for a long period, environmental contamination do not has a relevant role in bluetongue epidemiology. On the other hand, a favourable environment (puddles, wet-warm climate) is pivotal for the presence of BTV vectors.
1.1.5	*Seasonal cycles / outbreak influenced by climate anomalies*
1.2.5	Seasonal cycles can influence feeding and mating activities of biological vectors (adult females of *Culicoides*). These cycles are correlated to climate and are typically observed when the disease is present at northern latitudes (north of Italy, France, Germany, etc.). Climate anomalies, such as excessive rain and abnormal temperature peaks, may alter seasonal cycles and promote the diffusion of the vectors.
1.1.6	*Factors that facilitate the presence of the agent (scarce hygiene, biosafety, management, etc.)*
1.2.6	Poor levels of biosecurity. Inadequate control of animal transfers (areas at risk); inadequate control of biological vectors; favourable environment for vectors (wet-warm climate for feeding, presence of wet areas for mating); absence of a proper epidemiological surveillance; presence of susceptible species, amplifying hosts and biological vectors in the same area.

1.1.7	*Stability of the agent within the environment*
1.2.7	Neglectable because of the routes of transmission of the diseases. [The virus may persist within favourable environments for years. BTV is inactivated by low (<6) or high (>8) pH, high temperature (50°C for three hours or 60°C for 15 minutes) and proper disinfectants]
1.1.8	*Likelihood of eradication*
1.2.8	Possible. Likelihood of eradication is correlated to the presence of the vectors, amplifying hosts and wild hosts.
1.3	**Number of species involved**
1.3.1	*Number of species involved (specify which ones)*
	High. Sheep, goats, cattle, buffaloes, deer and domestic camelids. BTV infections are also described in dogs due to an accidental contamination of vaccines for pet.
1.4	**Speed of spread**
1.4.1	*Speed of spread within the farm*
	Variable (depending on control measures and vectors diffusion). In sheep bluetongue, without control measures, may spread very fast and morbidity can reach 100%.
1.4.2	*Speed of spread between farms*
	Variable (depending on control measures and vectors diffusion). In sheep bluetongue, without control measures, may spread very fast and morbidity can reach 100%.
1.4.3	*Likelihood of spread without hosts movement outside the farm*
	Variable (depending on control measures and vectors diffusion). In absence of effective control measures BTV can rapidly spread among farms without host movements throughout biological vectors. Biting midges of *Culicoides* spp. tend to live relatively near the area where they were born; however, winds may transport these vector for several kilometres. Some Authors speculated that, during the early 2000, BTV-8 were transported by the wind from North Africa to Sardinia and Balearic Islands (similar to sand carried by the wind to these island after sand storms in Africa).
1.5	**Vectors as *reservoir* and potential source of the disease**
1.5.1	*Disease cycle influenced by vectors*
	Yes, endemic areas and natural outbreaks are possible only where the vectors are present. Biting midges of the genus *Culicoides* act as BTV biological vector. Genus *Culicoides* includes over 1,400 species (almost all haematophagous) but only a minor fraction of these species can spread BTV (different species may also spread diverse BTV serotypes).
1.5.2	*Presence of the vectors within the regional / national territory*
	The vectors are present in Lombardy and in other parts of Italy (where climate is favourable). *C. imicola* is the characteristic vector of BTV of African origins and, before 1998, bluetongue outbreaks were related only to *C. imicola* presence. After 1998, BTV were also isolated from different species, belonging to *pulicaris* or *obsletus* complexes (although only 0.4% of individuals within these complex seems able to act as vector).
1.5.3	*Presence of the vectors influenced by peculiar areas / climates*
	The presence of the vector is correlated to warm temperature, high humidity and availability of wet areas for reproduction. Vectors are active (mating and feeding) during all the year in tropical areas, in temperate areas their activities follow seasonal cycles (favourable climate). Low temperatures have a negative impact on survival of the vectors; according to experimental data the vitality of *C. imicola* eggs begins to decrease after a week at 6.5°C and reach zero after 37 days; however, some adults (about 15%) may survive even at -1.5°C for 15 days.

1.5.4	*Vectors likelihood of survival, reproduction, spread the disease, act as reservoir*
	Culicoides spp. is able to colonize and survive in areas with favourable climate and environment. The vector, afterwards the blood meal, cannot infect the host before 7 to 10 days (intrinsic incubation period), after this period it will remain infectious during its entire lifespan. Life expectancy of the vector is usually brief, 10 to 20 days, however, some females midges may survive up to 90 days, and thus, they can have a large number of blood meals. The vectors are typically active from the dusk till the first hours of the dawn but their activities cease in case of adverse conditions (low humidity, temperature < 12°C, wind > 3m/s, rain, complete darkness). High temperatures promote BTV replication within the vector and reduce the intrinsic incubation period, as the temperatures decrease virus replication slows-down and incubation period increases (under 15°C the spread the disease by vectors tends to stop). The presence of the vectors is mandatory for BTV spread, nonetheless, there is no evidence of transovarial transmission of the virus in *Culicoides* spp..
1.6	**Risk of spread to susceptible species**
1.6.1	*Likelihood of transmission*
	High in absence of control (in unvaccinated sheep morbidity may reach 100% if vectors are not controlled).
1.6.2	*Route of transmission*
	Various route of transmission are reported: – Transmission by vector (adult females) only during the blood meal (primary source of spread). – Transmission to new-borns ruminants due to infected colostrum is described, however, the epidemiological relevance of this route of transmission is not clear. – Venereal transmission is possible in ruminants and, in cattle, transplacental transmission is also reported (BTV-8). – In dogs described cases of infection due to contaminated vaccines and in lynx via infected meat (BTV-8).
1.6.3	*Peculiar condition that influence transmission*
	Several condition can promote spread of the disease: – Poor biosecurity and lack of surveillance. – Poor control of movements of potential hosts. – Poor control of potential vectors. – Simultaneous presence of amplifying and sensible hosts within areas at risk. – Entrance of BTV in areas where the potential hosts are not immunised. – Temperature peaks and high humidity.
1.7	**Wildlife as reservoir and potential source of the disease**
1.7.1	*Species involved*
	High. Wild ruminants and other *Artiodactyla* (e.g. camelids) can be infected, however, these animals usually develop asymptomatic infections. Exceptions are: mouflons (mild symptoms and very low mortality), white-tailed deer (severe disease with haemorrhagic fever and high mortality), pronghorn antelopes (severe disease with haemorrhagic fever and high mortality), and New World camelids (mild symptoms and very low mortality). Seroconversion without symptoms is described in some wild carnivorous, and mortality is reported in Eurasian lynx due to ingestion of infected meat (BTV-8).

1.7.2	*wildlife / livestock and pet / human interactions*
	Epidemiological role of wildlife, especially in Lombardy and similar areas, is not well clear and further researches are needed (in some wild ruminant species in various areas of Africa and North America bluetongue may be considered endemic). BTV can infect red deer generally with mild to none clinical symptoms, however, infected deer present both viraemia and seroconversion. Recent studies identified reed deer as a potential *reservoir* of the disease in wildlife. Epidemiological roles of wild *Artiodactyla* (other than ruminants) and wild carnivorous are still unclear.
1.7.3	*Endangered species involved*
	Neglectable in Lombardy at the moment (potential future risks for lynxes, but unlikely).
1.8	**Potential for silent spread**
1.8.1	*Likelihood of recognition due to the clinical symptoms*
	Medium in case of acute infections in susceptive hosts, however, symptomatology may vary and clinical cases may be show symptoms similar to other diseases. In addition, in several species BTV infections can be asymptomatic. The name of the disease, bluetongue, originate from the dramatic blood flow disturbances to the tongue that may occur in some cases, nonetheless, this sign is not very common (severe infections only).
1.8.2	*Spread by subclinical / asymptomatic hosts*
	High risk (mild to asymptomatic in several species). BTV can circulate in blood associated to the membrane of red blood cells, and thus, the infection may present prolonged viraemia, delayed immune response, and improved transmission during the blood meal of the vectors. Cattle are the most important amplifying host because in this species viraemia can start 4 days after infection and persist till 60 days (according to OIE recommendations). Moreover, cattle do not show symptoms during the infection (except for BTV-8).
1.8.3	*Incubation time*
	On average 5 to 10 days (up to 20 days).
1.9	**Variability of the agent**
1.9.1	*Species / Types*
	One species, several serotypes. Bluetongue is caused only by Bluetongue virus, however, BTVs are high variable virus and there are 26 serotypes known worldwide, including BTV-25 (Toggenburg virus) and BTV-26 (serotype from Kuwait). BTV genome consists of 10 strands of double-stranded RNA that codify just as many proteins, 7 of these proteins are structural (VP1-7) and the other 3 are non-structural (NS1-3/3a). VP2 and VP5 are two highly variable proteins (especially VP2) that determinate viral serotype. Serogroup is manly defined by VP7.
1.9.2	*Mutations*
	Yes. Genetic shift is possible during co-infection with different BTV, reassortment of viral genes may also occur between vaccine and wild virus in case of simultaneous infection. Genetic drift is possible too and every RNA strand can drift independently.
1.9.3	*Number of hosts / vectors (host-specificity)*
	Low host-specificity. BTV is able to infect several ruminants species and other *Artiodactyla*. Seroconversion is reported in some African carnivorous. Deadly infections are described in lynxes and dogs due to particular conditions.

1.10	**Knowledge of host-pathogen interaction**
1.10.1	*Status of knowledge of host-pathogen interaction*
	Very good. Aspects of the host-pathogen interaction of particular interest: – Virus associated with the red cell membrane during viraemia (relevance in epidemiology). – Virus infection of endothelium (relevance in virulence). – Virus infection of monocytes and host immune response (relevance in diagnosis and virulence). Some aspects of cellular immunity are not yet clear and need further investigations.
1.11	**Knowledge of immunology**
1.11.1	*Complete / partial / absent knowledge of humoral immunity*
	Almost complete. Neutralising antibodies are particularly relevant in humoral immunity because they provide a long-lasting immunity against BTV infections of the same serotype. However, these antibodies do not protect against infection of different serotypes.
1.11.2	*Complete / partial / absent knowledge of cellular immunity*
	Good. Some aspects of cellular immunity are still unclear.
2	**SOCIO-ECONOMIC IMPACT**
2.1	**Impact on production within the region**
2.1.1	*Production losses*
	None in Lombardy, the region is bluetongue-free for the season. Production losses may significantly vary due to host species involved and BTV serotype. Economic impact of bluetongue can be dramatic in sheep, in cattle the disease is less severe and only BTV-8 may cause relevant production losses. In cattle BTV-8 can induce both direct and indirect losses. Direct losses: – Abortions, stillbirths, malformed calves. – Reduced fertility. – Reduced milk yield (milking cows) or reduced weight gains (beef cows) – Culling of infected animals and related cost (when mandatory because of the control plan) Indirect losses: – Loss of future income due to the culling of infected animal (particularly during the lactation and/or of for high-value bovine). – Routine expenses for cow with reduce/no production. – Costs of veterinary services (symptomatic therapy and diagnostic) when permitted – Ban of animal movements and trades (when present) Bluetongue in sheep can cause further losses because of the severity of the disease (morbidity up to 100% and mortality up to 70%).
2.1.2	*Quality losses*
	None in Lombardy, the region is bluetongue-free for the season. In case of outbreak: reduced weight gains (beef cows and sheep) and decrease of fleece quality (sheep, cutaneous lesions).
2.1.3	*Menace to survival of livestock industry*
	None in Lombardy, the region is bluetongue-free for the season (possible risks in the future). In Italy, during the firsts years of 2000, over 500,000 sheep were lost (death or culling) and animals trades were banned for a long time due to the most severe bluetongue epidemic in the history our Country. Lack of an efficient surveillance system may permit the introduction of the diseases into a “virgin” population and put in danger the related livestock industry.

2.2	**Economic impact of the control plan**
2.2.1	*Voluntary / mandatory control plan*
	None (Lombardy is seasonal free).
2.2.2	*Actual cost of the surveillance*
	Very Low (entomological surveillance, sentinel animal, surveillance in slaughterhouses)
2.2.3	*Outbreak within the region (per year)*
	None.
2.2.4	*Adopted control measures and actual costs*
	Not applicable.
2.3	**Potential economic direct impact**
2.3.1	*Production and movement limited / banned*
	Ban / limits in animals movements and trade in case of outbreak or area under restriction.
2.3.2	*Potential economic cost*
	High. In 2006, an epidemic of BTV-8 struck over 400 farms (cattle and small ruminants) in the Netherlands and caused economic losses for 32 million of euros; economic cost were distributed as 91% for control, 7% for diagnostic, and 2% for treatment. Bovine were the main source of these economic losses (88%), particularly milking cows represented the 55% of the costs (highest value per animal). In 2007, the epidemic of BTV-8 in the Netherlands spread to over 6,000 farms and economic costs were estimated in about 163 – 175 million of euros (92% as production losses); bovine were again the main source of these losses (85% of total costs).
2.3.3	*Control strategies available (vaccination and therapy / Test-and-cull / Stamping out)*
	At the moment the control is based on identification of areas at risk, vaccination, and ban/limitation of animal movements. Various control strategies are available: – Stamping out and zoning (high costs, low effectiveness). – Zoning without vaccination (costs depending on animal trades bans, low effectiveness). – Zoning with vaccination of small ruminants only (cost may vary, effectiveness depending on number of cattle within the restricted area). – Zoning with vaccination of all potential host (cost may vary, good effectiveness if immunisation is induced against the correct serotype).
2.3.4	*Costs of intervention (surveillance and control)*
	Low for surveillance. In the Netherlands, during the 2006-2007 epidemic, costs of surveillance were estimated in about 2 million of euros. High for control (culling and zoning). In the Netherlands, during the 2006-2007 epidemic, cost of control were estimated in about 22 (2006) and 11 million (2007) of euros.
2.4	**Potential economic indirect impact**
2.4.1	*Consequences on the distribution of the products*
	Ban of animal movements for long periods in areas where sheep have an important role in local economy may lead to both severe financial losses and social tensions, e.g. 2000-2002 epidemic in Italy. Epidemics of BTV-8 in North Europe during 2006 and 2007 caused relevant negative repercussion on products distribution.
2.4.2	*Market value losses*
	None indirect. Bluetongue is not a zoonosis, and thus, it never leaded to indirect market value losses due to fear fed by mass media.

2.4.3	*Nation-wide ban of distribution*
	None. Ban within restricted areas (zoning size may vary depending on epidemiological status).
2.4.4	*Cost of treatments and control of the disease in humans*
	Not applicable.
2.4.5	*Tourism losses and menace to biodiversity*
	None at the moment. Potential menace to vulnerable sheep breeds and lynxes. Severe risks in areas (not in Italy) where with-tailed deer and pronghorn antelopes are present.
2.4.6	*Restriction on the entire productive system*
	Potential risks in case of epidemic in "virgin" populations. At the moment EU laws permit, in particular conditions, animal movements even in restricted areas.
3	**IMPACT ON PUBLIC HEALTH**
3.1	**Relevance in laws (locals to international)**
3.1.1	*Relevance in laws (region / nation / EU / worldwide)*
	Not applicable.
3.2	**Zoonotic potential**
3.2.1	*Possibility of transmission between animals and humans (yes / no / unclear)*
	None (only one case reported due to a laboratory accident).
3.2.2	*Likelihood of transmission between animals and humans*
	Not applicable.
3.2.3	*Route of transmission from animals to humans (direct, indirect; vector, food, environment, airborne)*
	Not applicable.
3.2.4	*Species barrier*
	Not applicable.
3.2.5	*Virulence factors*
	Not applicable.
3.2.6	*Likelihood of underestimate human cases*
	Not applicable.
3.3	**Likelihood of occurrence**
3.3.1	*Probability of occurrence*
	Not applicable.
3.4	**Spread in humans**
3.4.1	*Likelihood of transmission between humans*
	Not applicable.
3.4.2	*Route of transmission between humans (direct, indirect)*
	Not applicable.
3.5	**Impact on human health**
3.5.1	*Severity of the disease*
	Not applicable.
3.5.2	*Symptoms duration and time off work length*
	Not applicable.
3.5.3	*Permanent damages*
	Not applicable.
3.5.4	*Mortality*
	Not applicable.

3.6	**Impact on food safety**
3.6.1	*Likelihood of infection / intoxication due to infected / contaminated food*
	Not applicable.
3.6.2	*Infectious / toxic dose*
	Not applicable.
3.6.3	*Mandatory precautions*
	Not applicable.
3.7	**Bioterrorism potential**
3.7.1	*Potential to cause substantial harm in humans*
	Not applicable.
3.7.2	*Agent availability*
	Not applicable.
3.7.3	*Facility of use and conservation (Labs / trained professionals / sole person)*
	Not applicable.
4	**IMPACT ON TRADE**
4.1	**Impact on regional trade due to current laws**
4.1.1	*Ban / Limitations on trade (positive animal only, herd, limited area, region-wide)*
	None at the moment (Lombardy is seasonal free). In case of outbreak ban of potential hosts movements (trade and grazing) within the restricted area (exception for culling).
4.1.2	*List of banned products*
	Semen, eggs and embryos not in compliance with EU standards (Commission Regulation (EC) No. 1266/2007 and following integrations/modifications).
4.1.3	*Free-status loss*
	Yes. At the moment Lombardy is declared seasonal free (in compliance with (EC) No. 1266/2007, DGSAFV III/4786 note of 03/13/2009, and DGSAFV 4575 note of 03/12/2010).
4.1.4	*Difficulty and time needed to regain free-status*
	High difficulty and prolonged times. In order to regain the free status BTV must be absent within the area for at least two years after the outbreak (Commission Regulation (EC) No. 1266/2007 and following integrations/modifications).
4.2	**Impact on national / EU trade due to current laws**
4.2.1	*Ban / Limitations on trade (positive animal only, herd, limited area, nation-wide)*
	Ban and limitations on trade between restricted areas and seasonal-free areas. However, EU laws still permit, in particular conditions, animal movements outside restricted areas (Commission Regulation (EC) No. 1266/2007 and following integrations/modifications).
4.2.2	*List of banned products*
	Semen, eggs and embryos not in compliance with EU standards (Commission Regulation (EC) No. 1266/2007 and following integrations/modifications).
4.2.3	*Free-status loss*
	Not applicable (at the moment Italy is not bluetongue free).
4.3	**Impact on international trade due to current laws**
4.3.1	*Ban / Limitations on trade (positive animal only, herd, limited area, nation-wide or EU-wide)*
	Ban and limitations on trade may vary (single animal/farm/whole geographical area) depending on epidemiological status of the zone, no limitation from free zones (OIE Terrestrial Animal Health Code - cap. 8.3).
4.3.2	*List of banned products*
	Semen, eggs and embryos not in compliance with OIE standards (OIE Terrestrial Animal Health Code - chap. 8.3).

4.3.3	*Free-status loss*
	Not applicable (at the moment Italy is not bluetongue free).
4.3.5	*Countries with particularly strict laws (about the disease)*
	None.
4.4	**Potential for zoning**
4.4.1	*Zone of control size*
	Zoning may vary: – In case of suspect: delimitation of an infected zone of 4 km around the farm source of the potential threat. – In case of confirmation by CESME (*Centro Studi Malattie Esotiche*): delimitation of a zone of control (20 km wide) and a zone of surveillance (entire province). Both these zones may be increased or decreased based on vaccination and epidemiological status.
5	**IMPACT ON ANIMAL WELFARE**
5.1	**Potential impact on animal welfare (duration)**
5.1.1	*Presence and duration of animal welfare damages*
	Variable (on average 8 – 10 days). BTV infections may vary greatly from asymptomatic forms to acute severe diseases (depending on viral serotype, host species, host breed). Severe acute infections can lead to death in 8 – 10 days (48 hours in peracute cases) or, if the host survive (and not be culled), recovery takes may require months.
5.2	**Potential frequency of severe distress**
5.2.1	*Percentage of animal with severe distress*
	Very high, morbidity up to 100% in some sheep breeds. In sheep mortality is usually high and may vary from 30% to 70%; in cattle only BTV-8 is able to induce clinical symptoms but mortality is low (< 1%). (In white-tailed deer and pronghorn antelopes fatality rate may reach 90%)
5.3	**Severity / reversibility of the disease**
5.3.1	*Severity of the disease and reversibility of the damages*
	Variable (very high in susceptible hosts). Severity of the BTV infections may vary greatly, from asymptomatic infections to severe diseases with permanent damages and high mortality. Outcomes of the infection are influenced by BTV serotype, host species, and host breed (European sheep breeds, such as Merino, are the most susceptible). In sheep: peracute infections induce pulmonary oedema and rapidly lead to death; acute infections may cause fever, depression, anorexia, head oedema, dyspnoea, severe blood flow disturbances (skin and mucosae), nasal discharge (from serum to pus), excessive salivation, ulcerations, cyanosis, haemorrhages, aborts, various gastro-enteric disturbances, foot lesions (hyperaemic coronary bands, lameness), and torticollis. In cattle: BTV infections are usually asymptomatic, however BTV-8 may induce clinical diseases with symptoms similar, but less severe, to sheep infections and low to no mortality. Moreover, BTV-8 can easily infect foetus and can cause cerebral malformation and death. In dogs: severe infections of bluetongue had been reported after an accidental contamination of dogs vaccines; clinical signs were pneumonia and vasculitis, the fatality rate was very high in pregnant bitches.
5.3.2	*Available therapies and their effectiveness*
	None. Effective drugs against BTV do not exist, only sustain and symptomatic therapies are available.

5.4	**Impact on animal freedom**
5.4.1	*Potential restriction on the "Five Freedom" (freedom from fear and distress NOT included)*
	Potential restrictions to: – Freedom from hunger and thirst (erosions and ulcerations in the mouth, atrophy of the muscles). – Freedom from discomfort (foot lesions, bites from infected midges in absence of control). – Freedom from pain, injury or disease. – Freedom to express normal behaviour (foot and perineal lesions).
6	**CONTROL TOOLS**
6.1	**Proper tools for diagnosis**
6.1.1	*Validated kits availability within the nation*
	Experimental Zooprophylactic Institute of Lombardy and Emilia Romagna (IZLER) provide the following diagnostic services: – PCR qualitative and real-time PCR; – Competitive ELISA (VMRD kit); – Competitive ELISA (IZSAM kit); – ELISA for antibodies in serum or milk (ID VET kit).
6.1.2	*Laws that rule the surveillance*
	Surveillance plan, tools for diagnosis, and outbreaks identifications must be in compliance with EU laws (Council Directive 2000/75/EC of 20 November 2000, Commission Regulation (EC) No. 1266/2007, and following integrations/modifications).
6.1.3	*Techniques described by international organisation (OIE, WHO, UE, etc.)*
	OIE MANUAL OF DIAGNOSTIC TESTS AND VACCINES FOR TERRESTRIAL ANIMALS (Chap. 2.1.3): d) <u>Identification of BTV</u> – *Virus isolation* (from blood or other tissues, prescribed tests for international trade): o Isolation in embryonated hens' eggs (about 1 week required for identification, good sensitivity but complicated). o Isolation in cell culture (about 5 days but low sensitivity). o Isolation in sheep (up to 28 days, most sensitive technique but also most costly). – *Immunological methods*: o Identification of serogroup (immunofluorescence, antigen capture enzyme-linked immunosorbent assay, immunospot test). o Identification of serotype (virus neutralisation). – *Polymerase chain reaction techniques*: o Reverse-transcription polymerase chain reaction (prescribed tests for international trade), fast and sensitive but risks of false positive due to contamination (by other samples or primers) and false negative due to incorrect sampling or wrong primers. o Real-time reverse-transcription polymerase chain reaction tests (not yet validated). e) <u>Serological tests</u> – *Complement fixation* (outdated since 1982) – *AGID* (an alternative test for international trade): problems due to low specificity (detect both anti-BTV and anti-EHDV antibodies) and subjectivity exercised in reading the results. – *ELISA techniques*: o Competitive ELISA (an alternative test for international trade), fast and good specificity. o Indirect ELISA (bulk milk), only for surveillance purposes.
6.1.4	*DIVA test possibility / obligation of use*
	None

6.1.5	*Overall judgement on tools for control*
	Tools for surveillance and control in compliance with EU standard are considered effective for safe trades of sensible animal species, semen, eggs and embryos. Vaccination provide a strong and effective immunity against BTV. However, vaccination presents also relevant drawbacks such as: risks of severe adverse reaction, reassortment between vaccine and wild-type BTV, reversion to virulence and infections in unvaccinated animals, absence of cross-protective immune response against different serotypes (introduction of a diverse wild-type BTV serotype within a population may lead to an epidemic). Lombardy region, at the moment, is a low-risk region, nonetheless, an effective surveillance plan is still pivotal. Several areas within the region provide favourable climate and ecology for *Culicoides* spp. seasonal activities (feeding and mating). Moreover, the wind may transport infected midges from high-risk area to Lombardy and increase the level of risk in the region (particularly if a new serotype is introduced in Italy).
6.2	**Proper tools for prevention (within the region/area)**
6.2.1	*Obstacles / incentives to prevention*
	Obstacle: control of the vectors may be difficult, frequent animal movements (trade and grazing), high densities of potential hosts and farms, unclear epidemiological role of wildlife, illegal trade of potential hosts in region (from high-risk areas), introduction of new serotypes in Italy. *Incentives*: surveillance and prevention are essential in bluetongue control, EU incentives and grants, prevention of an outbreak prevent also economic losses due to the diseases and ban of animal movements.
6.2.2	*Available prevention strategies and their efficacy*
	Various strategies can be adopted: high levels of biosecurity, control of the vectors, and surveillance. Namely, control of animal movements, reduction of contacts between amplifying and sensitive hosts, vaccination, control of semen and embryos, insecticides and midges repellents (temporary effective only), protection of the potential hosts during the hours of midges activities (midges net). Various strategy for surveillance are available and can be used in association (combinations may vary depending on epidemiological status): – Clinical surveillance: all the suspect cases must be reported. – Serological surveillance: sentinel animals (usually cattle); single animals tested on farm and slaughterhouse, bulk milk tests. – Entomological surveillance: fixed and mobile traps to verify presence, diffusion, seasonal cycles, and infective status of potential vectors. – Virological surveillance: Virus isolation and typing.
6.2.3	*Commercial vaccines availability in EU / Worldwide*
	Several vaccines, attenuated or killed, are available within EU and Worldwide. Additionally, recombinant vaccines are under development.
6.2.4	*Marker vaccines availability in EU / Worldwide*
	None.
6.2.5	*Vaccination efficacy*
	Vaccination presents both advantages and disadvantages. *Advantages*: strong and prolonged immunity that provide protection against clinical disease (especially modified live vaccines [MLV]), massive vaccination of both cattle and small ruminants is possible, mixture of different serotypes available, relatively easy integration into a control plan, may be simplify animal trade. *Disadvantages*: relatively common adverse reaction (0.1% in cattle and 0.5% in small ruminates but overestimated according some Authors), absence of cross-protective immune response against serotypes different from vaccine ones, risks of reassortment between vaccine and wild-type BTV (risks can be significantly lowered if vaccination is not provided during the seasonal activities of the vectors), infections of MLV in unvaccinated animals (risks can be significantly lowered if vaccination is not provided during the seasonal activities of the vectors), in MLV risks of excessive/inadequate

	attenuation (protective cellular immune response is proportional to BTV replication within the host), reversion to virulence (unlikely for some Authors). In addition, some problems may arise due to the presence of only one producer of BTV vaccines in Italy (natural monopoly, supply shortages).
6.2.6	*Laws that rule vaccination*
	Compulsory vaccination, in case of zoning, in compliance with Italians and EU laws (Commission Regulation (EC) No. 1266/2007, DGSAFV III/4786 note of 03/13/2009, DGSAFV 4575 note of 03/12/2010, and following integrations/modifications). Lombardy region is seasonal free, the last mandatory vaccination campaign was in Mantova province during 2009 due to an outbreak of BTV-8 in Veneto region ("*decreto direzione generale sanità n. 7209 del 02/07/2008*").
6.3	**Proper tools for control (within the region/area)**
6.3.1	*Obstacles / Incentives to control*
	Obstacle: control of the vectors may be difficult, frequent animal movements (trade and grazing), high densities of potential hosts and farms, unclear epidemiological role of wildlife, illegal trade of potential hosts in region (from high-risk areas), introduction of new serotypes in Italy. *Incentives*: mandatory control plan, EU incentives and grants, extinguish of an outbreak prevent future economic losses due to the diseases and stops the ban of animal movements.
6.3.2	*Available control strategies and their efficacy*
	Various strategies can be adopted: high levels of biosecurity, control of the vectors, and a control plan. During the last 15 years different control strategies, based on zoning, were proposed such as: stamping out, modified stamping out, vaccination, or ban of potential hosts movements. However, these strategies, when used alone, showed poor effectiveness. The actual control system encompasses a surveillance plan (epidemiological and entomological) and control measures in case of outbreak, such as zoning (area under control + area under surveillance) and massive vaccination of potential hosts (domestic species only). This approach provide good effectiveness when used to control a known serotype but it has some limits. Indeed, vaccination do not provide cross-immunity against different serotypes and vectors, harbouring a diverse serotype, may be transported by wind from neighbour Mediterranean Countries where effective surveillance systems are not present. Lombardy, at the moment, is seasonal free and a low-risks zone, nonetheless, this scenario may change in future because of several areas within the region provide, during the warm season, favourable environment for *Culicoides* spp.. Therefore, maintaining an effective surveillance system is essential.
6.3.3	*Laws that rule control strategy*
	Control tools must be in compliance with EU laws (Council Directive 2000/75/EC of 20 November 2000, Commission Regulation (EC) No. 1266/2007, and following integrations/modifications).
6.4	**Proper tools for therapy**
6.4.1	*Therapeutic protocol in use (cure and prophylaxis)*
	Effective drugs against BTV do not exist. Only sustain therapies, vaccines, and midges repellent are available.
6.4.2	*Laws that rule therapies*
	Not applicable.
6.4.3	*Residual risks / suspension time*
	Not applicable.

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Appendix D: Systematic review results

The results of our systematic review are collected and sorted by pertinence in the following table:

Score = 0 (*no pertinence*)

1. **Abebe R. & Wolde A.** (2010). A cross-sectional study of trypanosomosis and its vectors in donkeys and mules in Northwest Ethiopia. *Parasitology Research, **106,*** 911-916.
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Score = 1 (*low pertinence*):

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Appendix E: Scientific papers

Risk Prioritization as a Tool to Guide Veterinary Public Health Activities at Regional Level

Authors: Scali F.[1], Bonizzi L.[1] , Ferrari N.[1], Ferrero F[1], Frazzi P.[2], Grilli G.[1], Lanfranchi P.[1], Mortarino M.[1], Sala V[1], Taloni D[1] & Zecconi A.[1]

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Abstract

A model for prioritization and risk characterization focused on zoonosis and food safety was developed for diseases of interest in veterinary public health at a regional level. A previous model (DISCONTOOLS) based on scorecards was used as a basis to develop the new one . Formalized Consensus Process approach involving academics and veterinary officers was used to develop form, scorecards and relative guide. Scorecards were filled following available data, literature and expert opinions. A scorecard with maximum theoretical score of 1,000 was developed; it includes several areas of interest, with different categories and coefficient of importance. The following areas were identified: relevance of the disease, socio-economic impact, impact on public health, impact on trade, impact on animal welfare, control tools. A guide and a form were settled in order to fill the scorecard. From an initial list of 38 disease, 23 were scored. Among bovine diseases mastitis (*S. aureus*) showed the highest score; among small ruminants Query fever was the highest, among swine diseases the highest was salmonellosis, while among other animal diseases toxoplasmosis had the highest score.

This approach is conceived to aid professionals in risk prioritization, decision-making and to improve disease control systems at a regional level. It also allows to perform risk characterization in different backgrounds and to identify lacks of data in specific areas of interest for the diseases considered.

***Staphylococcus aureus* virulence factors in evasion from innate immune defenses in human and animal diseases.**

Authors: Zecconi A.[1] & Scali F.[1]

Immunology Letters 2013 Feb;150(1-2):12-22. doi: 10.1016/j.imlet.2013.01.004. Epub 2013 Jan 31.

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Abstract

In the last decades, *Staphylococcus aureus* acquired a dramatic relevance in human and veterinary medicine for different reasons, one of them represented by the increasing prevalence of antibiotic resistant strains. However, antibiotic resistance is not the only weapon in the arsenal of *S. aureus*. Indeed, these bacteria have plenty of virulence factors, including a vast ability to evade host immune defenses. The innate immune system represents the first line of defense against invading pathogens. This system consists of three major effector mechanisms: antimicrobial peptides and enzymes, the complement system and phagocytes. In this review, we focused on *S. aureus* virulence factors involved in the immune evasion in the first phases of infection: TLR recognition avoidance, adhesins affecting immune response and resistance to host defenses peptides and polypeptides. Studies of innate immune defenses and their role against *S. aureus* are important in human and veterinary medicine given the problems related to *S. aureus* antimicrobial resistance. Moreover, due to the pathogen ability to manipulate the immune response, these data are needed to develop efficacious vaccines or molecules against *S. aureus*.

How are important targets in development of S.aureus mastitis vaccine?

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Research in Veterinary Sciences (submitted)

Staphylococcus aureus represents one of the leading causes of mastitis in dairy cows worldwide. In cattle, *S. aureus* intramammary infections (IMI) may entail severe economic losses even without clear clinical signs. *S. aureus* IMI have variable outcomes due to virulence of the strain involved, immune defences of the host, different management choices, and environmental conditions. Management and environment can also induce selective pressure on *S. aureus* and amplify differences among farms. Furthermore, *S. aureus* IMI may pose a threat to public health as potential source of food poisoning or methicillin-resistant *S. aureus* (MRSA). *S. aureus* infections can be exacerbated by its several virulence factors and its ability to resist to many antibiotics. All these characteristics facilitate the onset of chronic mastitis and a successful treatment during milking is unlikely. Therefore, infected cows have improved cure rates during the dry period, otherwise, they should be culled. Improvements of management and udder health seem to have reduced prevalence of *S. aureus* IMI in several countries but the pathogen is still widespread and eradication, at this point, is improbable. The difficulty in eradication and the increasing concerns on antibiotics usages underscore the interest in developing new tools to control *S. aureus* mastitis. Over the last 40 years, vaccination has represented one of the most studied of these tools but, to date, no vaccine seems to provide reliable protection. This review will summarize current knowledge on the major vaccine targets, including surface proteins, capsular polysaccharides, biofilm, and toxins. Finally, we will discuss the present status of vaccination against *S. aureus* and the future of vaccines designs, including how difference among *in vivo* models may influence vaccines development.

Pattern characterization of genes involved in non-specific immune response *in Staphylococcus aureus* isolates from subclinical mastitis

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Research in Veterinary Sciences (submitted)

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Abstract

Staphylococcus aureus isolated from mammary gland are characterized by different genetic patterns, and this variability is behind the differences observed in both clinic and economic aspects of these infections. These bacteria have an impressive arsenal of virulence factors which facilitate invasion, improve adhesion to the host, promote immune evasion and impairs host defences. Ninety four isolates from 33 dairy herds were analyzed by the means of a microarray to investigate S. aureus virulence patterns and, in addition, the role of genes specifically involved in immune evasion. None of the 94 isolates considered were MRSA. However, 50% of the isolates belonged to complexes related to MRSA and to human diseases (CC1, CC5, CC8, CC20, CC398), while only about 25% of them can be considered as exclusively of bovine origin. The distribution of clonal complexes and the different gene patterns observed confirmed the presence of an influence of geographical localization, which has important implications both from *S. aureus* epidemiology and prevention.

The assessment of the influence of genes related to immune evasion on quarter milk cell count (SCC) gave some unexpected results. A low frequency of enterotoxin genes was observed, supporting the hypothesis that enterotoxins did not play a major role in bovine mastitis pathogenesis. Among the other genes, four of them (ssl7, ssl11, chip and cap8) showed to be significantly associated to an increase quarter milk SCC. These genes could be potential target for developing new vaccines against *S. aureus*.