



Radioscopy laboratory diagnostic of epizootic diseases in Belgium and European countries



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Introduction

Belgium and other European neighbouring countries faced several **emerging and re-emerging diseases** as well as zoonotic diseases over the last decade. However, it has been noticed that during these episodes adequate laboratory **diagnostic capabilities** were the clue to rapidly control and eradicate these diseases. An updated crisis scenario book, as it is implemented in the Belgian national laboratory (NRL), has showed all its usefulness during these situations.

Belgium plays a key role in international animal trade making it very sensitive to disease introduction via different pathways e.g. trade of live animals or animal products from around the world or via wildlife (translocation). It is therefore **crucial to react quickly** to these diseases, to establish appropriate contingency plans and to develop reliable diagnostic tests. Moreover, only few publications regarding the increase of diagnostic capacities are available in the scientific literature.

Materials and methods

Based on 29 selected diseases (Bianchini J., personal communication), a mapping of existing techniques was created. An overview of **assays available** and which would be used in different scenarios (risk importation, early detection, spread and freedom of disease) in national and international laboratories was established. If gaps were detected, international laboratories were contacted in order to create future partnerships. These data were completed by the analysis of the **World Animal Health Information System (WAHIS)** online tools of the World Organization for Animal diseases (OIE) for the 40 major European countries (possibilities and assays used) encoded for 23 of the 29 diseases included in the OIE list.

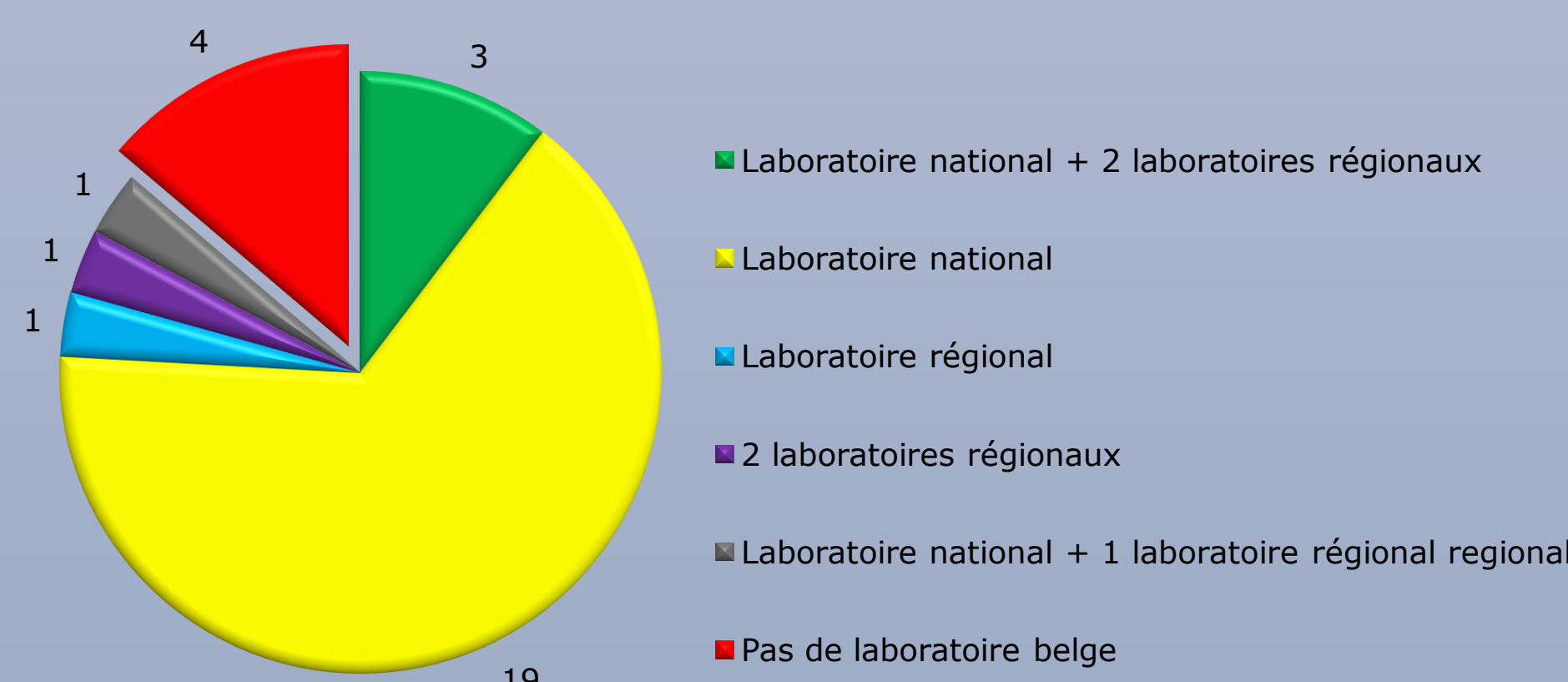
Results and perspectives

Using LimeSurvey® web application and the WAHIS online tools, a **large survey** in Belgium and in Europe has been completed to implement a specific database and to determine laboratory capacities in Europe. Using **four scenario analyses** (risk of importation, early detection, spread and freedom of disease) diagnostic flow charts and questions, concerning kits characteristics (e.g. sensibility & specificity), as well as additional questions regarding laboratory capacities, the experience and the degree of accreditation were asked. All data were gathered online in **decision trees** using Cmap® software. Also bilateral partnerships (techniques and/or collaboration protocols) between Belgian NRL and other European institutions will be developed.

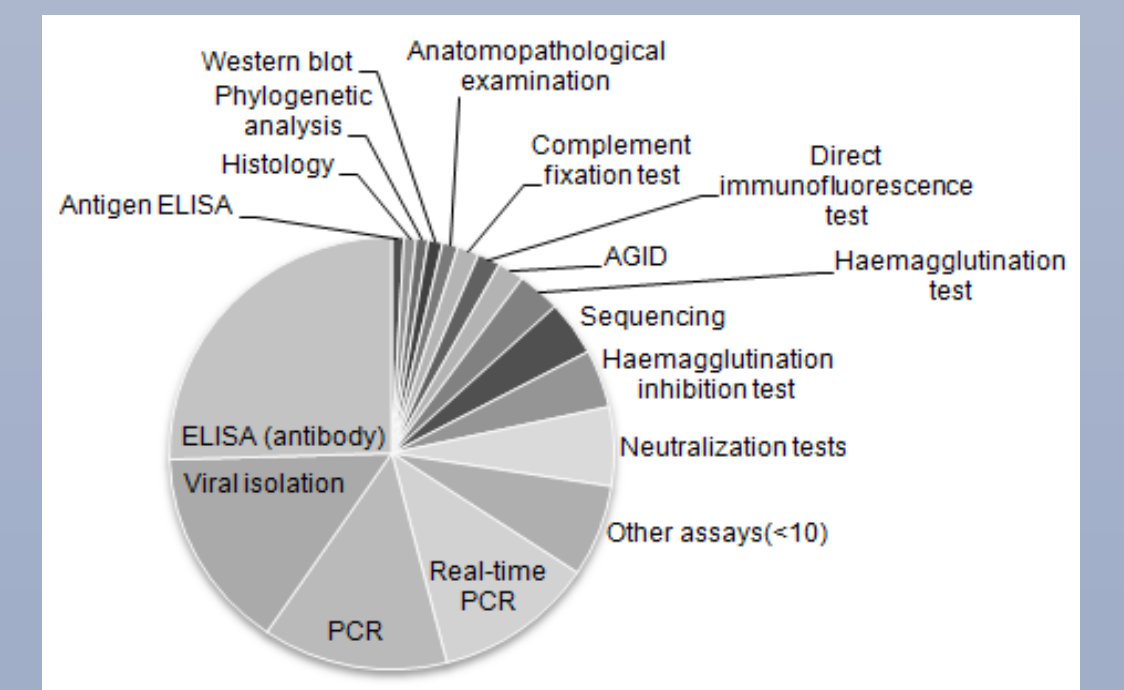
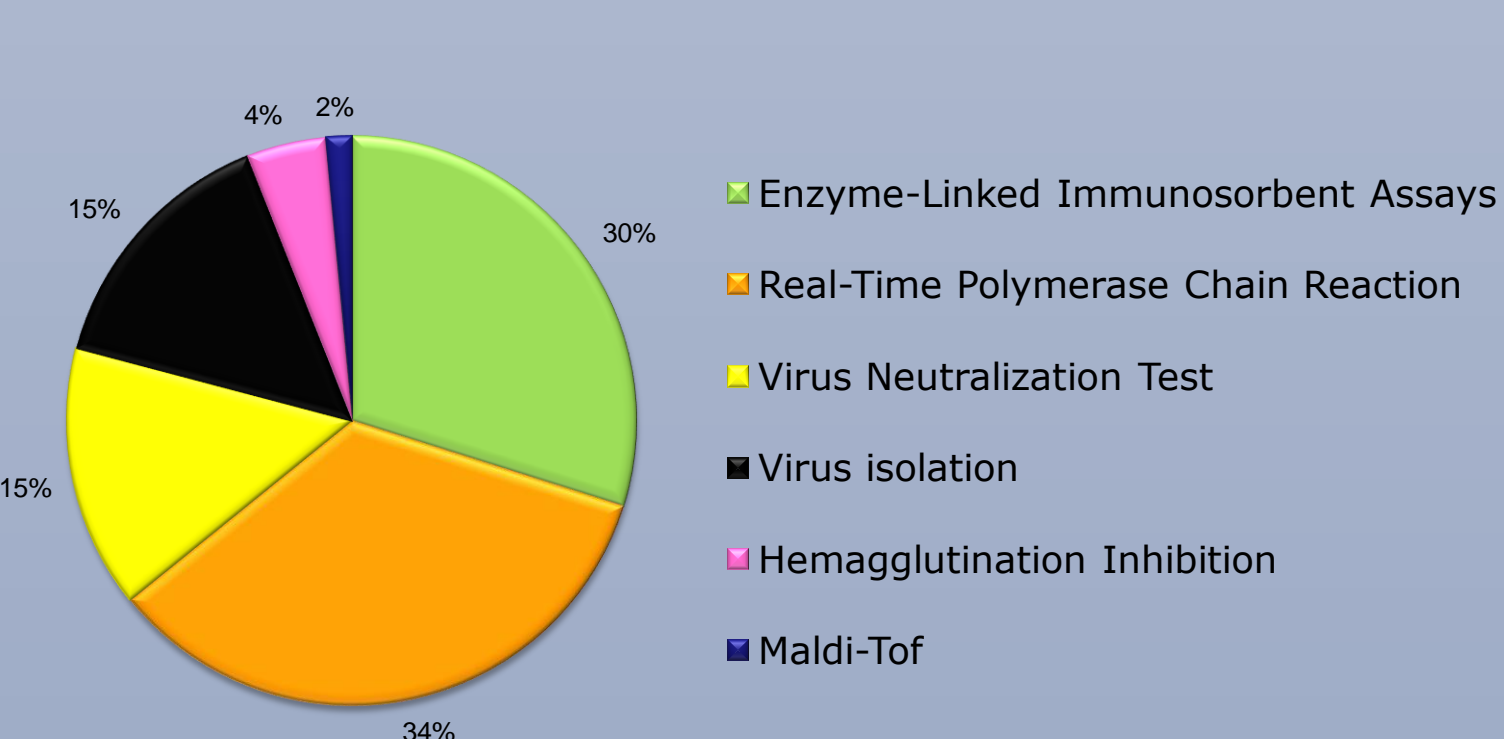
Example of a generic decision tree



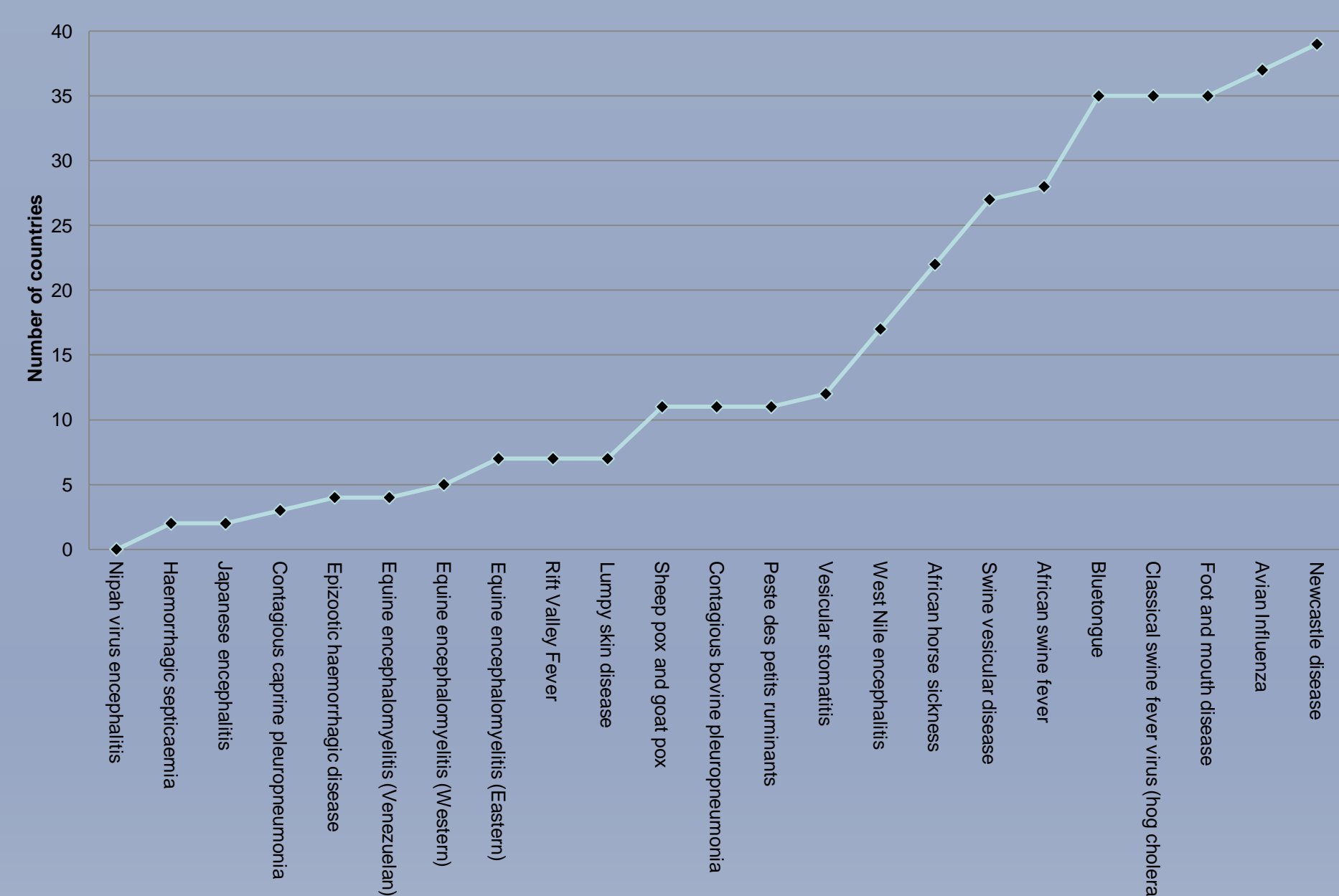
Number of diseases detected in routine in the national and regional Belgian laboratories



Frequency of the different assays used in the Belgian laboratories (fig. 2a) and in Europe (fig. 2b) to detect the selected diseases



Sum of possibilities of detection for the 23 selected diseases in the 40 European countries encoded in WAHIS



Possibilities of detection for the 29 selected diseases in 6 neighbouring countries of Belgium

| | Microbioculture | Microbiological | Amino acids | Nipah | Aino disease | Bluetongue | Cockle body fever | Classical swine fever virus (hog cholera) | Contagious bovine pleuropneumonia | Contagious equine pleuropneumonia | Epizootic haemorrhagic disease | Epizootic haemorrhagic fever (EHEC) | Epizootic haemorrhagic fever (EHEC) | Foot and mouth disease | Haemorrhagic septicaemia | Japanese encephalitis (JE) | Lumpy skin disease | Neuroleptospirosis | Nipah virus encephalitis | Pink eye and pink tongue | Porcine circovirus disease (PCVD) | Porcine circovirus disease (PCVD) | Rift Valley fever | Swine vesicular disease | Swine vesicular disease | Venezuelan equine encephalomyelitis (VEE) | Viral haemorrhagic septicemia | Viral haemorrhagic septicemia | Viral haemorrhagic septicemia | | |
|---|-----------------|-----------------|-------------|-------|--------------|------------|-------------------|---|-----------------------------------|-----------------------------------|--------------------------------|-------------------------------------|-------------------------------------|------------------------|--------------------------|----------------------------|--------------------|--------------------|--------------------------|--------------------------|-----------------------------------|-----------------------------------|-------------------|-------------------------|-------------------------|---|-------------------------------|-------------------------------|-------------------------------|--|--|
| Belgian laboratories | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| National laboratories | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Regional laboratories 1 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Regional laboratories 2 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Other laboratories (notified) | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| European laboratories | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Country 1 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Country 2 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Country 3 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Country 4 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Country 5 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Country 6 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| United States of America (2 laboratories) | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |

These surveys have showed that **virus isolation** and **virus neutralization test** are sensitive and specific and are therefore often considered as gold standard confirmation tests. However, they are slow, laborious and require biosafety laboratory facilities. For many diseases, **enzyme-linked immunosorbent assays (ELISA)** are often used and commercially available. Although its rapidity, often good sensitivity and specificity, ELISA needs to be standardized and fit for purpose which requires training and experience. Molecular methods such as the **polymerase chain reaction (PCR or mostly real-time PCR)** are very specific and sensitive which give qualitative and/or quantitative result in a few hours but need adequate material (e.g. good extraction protocol). In addition, PCR's are very often sequence-dependent and thus sensitive to mutations and contaminations which can lead to false negative results. **Sequencing** offers promising solutions but is currently only used as confirmation for first suspicious samples and is still restrained for research purposes because of the price for the equipment, the samples preparation and the requirement for trained staff.

On the other hand, this project suggests the importance of implementing, keeping up-to-dated online data at European level for (re-)emerging diseases, especially for Cache valley virus, Aino disease, Nipah disease, Porcine deltacoronavirus.

In conclusion, it was showed that Belgium is able to establish a diagnostic for 25 of the 29 of targeted diseases and underlined the importance of **collaboration** between laboratories permitting Belgium to have the diagnostic capacities to face all situations mentioned in the scenarios.

This work will help the Veterinary Authorities to take a faster, precise and well documented decision in case of an epidemic in Belgium.

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