DR. MICKAEL CARGNEL (Orcid ID : 0000-0002-7490-7903)

DR. SARAH AL WELBY (Orcid ID : 0000-0003-3217-9490)

PROF. CLAUDE SAEGERMAN (Orcid ID : 0000-0001-9087-7436)

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Improving laboratory diagnostic capacities of emerging diseases using knowledge mapping

Short running title: Knowledge mapping & laboratory diagnostic capacities

Cargnel Mickaël[1,2], Bianchini Juana[2], Welby Sarah[1*], Koenen Frank[1], Van der Stede Yves[1,3#], De Clercq Kris[1], Saegerman Claude[2]

[1]Sciensano, Brussels, Belgium

[2]Research Unit in Epidemiology and Risk Analysis applied to veterinary sciences (UREAR-ULiège), Fundamental and Applied Research for Animal and Health (FARAH) Center, Faculty of Veterinary Medicine, Liège, Belgium

[3]European Food Safety Authority, Parma, Italy

Corresponding author

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Claude Saegerman, University of Liège, Research Unit in Epidemiology and Risk Analysis applied to veterinary sciences (UREAR-ULiège), Fundamental and Applied Research for Animal and Health (FARAH) Center, Quartier Vallée 2, avenue de Cureghem 7A, B42, 4000 Liège, Belgium; Tel: +32 (0)43664579; Fax: +32(0)4 366 45 61; E-mail: claude.saegerman@uliege.be.

Disclaimer

*Sarah Welby is currently employed GlaxoSmithKline Vaccines. The positions and opinions presented in this article reflect the work carried out during her employment at Sciensano at the time of the study conduct and are not intended to represent the views or scientific works of GlaxoSmithKline.

#Yves Van der Stede is currently employed with the European Food Safety Authority (EFSA) in the ALPHA Unit that provides scientific and administrative support to EFSA's scientific activities in the area of Animal Health and Welfare. The positions and opinions presented in this article are those of the authors alone and are not intended to represent the views or scientific works of EFSA.

ABSTRACT

Over the last decade, European countries faced several emerging and re-emerging animal diseases as well as zoonotic diseases. During these episodes, the laboratory diagnostic capabilities were a key factor to rapidly control and/or eradicate them. Because of the associated socio-economic and health consequences, it is crucial to react rapidly and efficiently, not only during crisis but also in peacetime (i.e. preparedness). However, to date, there is no published method to identify diseases with diagnostic gaps and to prioritise assays to be implemented. This study was conducted based on the outcome of a prioritisation exercise in which 29 epizootic and exotic diseases with high risk of emergence or re-emergence in Belgium (Bianchini *et al*., 2020) were listed. Knowledge mapping was used to visualise and identify gaps in the diagnostic procedures for different epidemiological scenarios at national level. To fill these gaps, an overview of diagnostic capabilities at national and international level (laboratories and kits providers or manufacturers) as well as the published assays in the scientific literature and the prescribed assays by international institutions and kits providers was carried out. The outcome of this study revealed the usefulness of knowledge mapping as a tool to identify gaps and ultimately gain insight on alternatives for better preparedness and responsiveness While this exercise was limited to Belgium, we believe this exercise can benefit other countries and thereby enhancing knowledge sharing and collaboration to increase diagnostic capabilities for a common list of (re-)emerging diseases in crisis situation.

Keywords: Epizootic diseases; Emerging diseases; Diagnostic; Laboratories; preparedness; Capacity; Prioritisation; Knowledge mapping.

1. INTRODUCTION

An emerging disease can be defined as a disease with an incidence that has been significantly increasing compared to its baseline rate, in a determined population and region, and over a determined period of time (Toma and Thiry, 2003). A re-emerging disease can be defined as previously contained emerging disease that is re-emerges again.

Most recent pandemics, such as human immunodeficiency virus infection and acquired immune deficiency syndrome (HIV/AIDS), severe acute respiratory syndrome, and pandemic influenza, are caused by zoonotic pathogens originating from wildlife and for which the emergence was driven by ecological, behavioural, or socioeconomic changes (Morse et al., 2012; Bianchini et al., 2020). International experts of the World Organisation for Animal Health (OIE), the World Health Organization (WHO) and the United Nations for Food and Agriculture Organization (FAO) estimate that the risk of (re)-emergence of pathogens has reached an importance and a global impact never observed before (King, 2008; Vallat, 2016) and seems to increase (Anonymous, 2009; Jones *et al*., 2008). Indeed, continuing changes in socioeconomic, behavioural, commercial, environmental (e.g. deforestation and the decrease/lack of biodiversity, climate change and its consequences like global warming and severe rainfalls), political factors and the increase in international travels contribute to an amplification of environmental hazards and spread of exotic and (re)-emerging diseases (Rodriguez-Prieto *et al*., 2015; Gebreyes *et al*., 2014; Souza Monteiro *et al*., 2012). Additionally, the world animal population has been constantly increasing because of a rise in global demand for animal products. The majority of this need is located in less developed regions (Gebreyes *et al*., 2014) and are thus considered as hotspots for diseases emergence (Jones et al., 2008). Spatially explicit models can be used to identify regions most likely to establish the next emerging zoonoses (so-called hotspots of emerging infectious disease) which are located in regions where human activities take place against a background of high wildlife biodiversity, with concomitant microbial biodiversity (Morse *et al*., 2012). The tropical origin of those diseases could be considered as a threat for the northern hemisphere (Reviriego Gordejo *et al*., 2009) and should be monitored to prevent and rapidly deal with outbreaks (Morse *et al*., 2012).

Conventional surveillance system for re-emerging diseases is based on passive (clinical) detection combined with active sampling. However, conventional passive surveillance on limited number of samples is restricted by the diagnostic accuracy, visibility and specificity of clinical signs. Active surveillance on random sample surveys is highly expensive and timeless is a limiting factor (Rodriguez-Prieto *et al*., 2015). Therefore, differential diagnosis based on laboratory testing for a broad range of diseases is an asset for the identification, control and eradication of the pathogens (Elbers *et al*., 2014). It is an evidence that well-trained laboratories with adequate capacities and

quality assays are key factors to rapidly diagnose, control and/or eradicate any pathogen (Koenen *et al*., 2007; Muir-Paulik *et al*., 2016; Vallat, 2016; Westergaard, 2008). However, the OIE pointed out that rapid detection of emerging diseases could be improved in developing countries and even in some industrialized countries which suffer from deficiency in infrastructure, laboratory capacity and veterinary proficiency (Vallat, 2016).

The European legislation harmonized contingency plans for several highly transmissible diseases (e.g. European Regulation (EU) 2016/429) but left Member States (MS) free to prioritize and implement control strategies. Few scientific articles have focussed on the evaluation of laboratory capabilities to tackle (re)emerging diseases (Muir-Paulik *et al*., 2016) which could leave policy-makers without scientifically sound ideas of what they could and should actually strengthen (Savigny and Taghreed, 2009). Knowledge mapping, a collection of concepts structured hierarchically and populated with additional resources that explicates the concepts in a map (Coffey, 2015), could be useful to provide an overview of the laboratory capabilities and help for laboratory diagnostic gaps. Two dimensions might be considered to define a lack in diagnostic: missing adequate testing procedure at the hand, availability and preparedness of diagnostic resources when confronted to an epidemic.

This study will first review the diagnostic capabilities in Belgium (as an example) for a list of selected (re)emerging -26 viral and 3 bacterial- diseases recently published (Bianchini *et al*., 2020). It will then use knowledge mapping to visually identify major and minor gaps(s) in diagnostic in frequent epidemiological scenarios. An overview of diagnostic capabilities at international level (laboratories and kits providers/manufacturers) as well as the published assays in the scientific literature and the prescribed assays by international institutions will help to find collaborations and fill the gaps if necessary.

2. MATERIAL AND METHODS

2.1. List of diseases

For this study, 29 livestock diseases considered as emerging and reemerging disease were used. These diseases were selected from a previous published study and ranked according to risk of emergence in

Belgium using a multi-criteria decision analysis approach (Bianchini et al., 2020). These diseases were African horse sickness, African swine fever, Aino disease, akabane disease, avian influenza, bluetongue, Cache Valley virus, classical swine fever virus, contagious bovine pleuropneumonia, contagious caprine pleuropneumonia, epizootic haemorrhagic disease, equine encephalomyelitis (eastern, western, Venezuelan), foot-and-mouth disease, haemorrhagic septicaemia, Japanese encephalitis, lumpy skin disease, Newcastle disease, Nipah virus encephalitis, porcine epidemic diarrhoea, porcine deltacoronavirus (novel swine enteric coronavirus disease), Rift Valley fever, peste des petits ruminants, Schmallenberg disease, sheep pox and goat pox, swine vesicular disease, vesicular stomatitis, West Nile encephalitis.

2.2. Inventory of the Belgian diagnostic possibilities and capacities for three epidemiological scenarios: input for knowledge mapping

The need for diagnostic capabilities was assessed according to three classical epidemiological scenarios, which are: the early detection of disease, the characterisation of disease spread and the substantiation of freedom of disease as defined by the manual of diagnostic tests and vaccines for terrestrial animals from the OIE (http://www.oie.int/en/standard-setting/terrestrial-manual/access-online/).

A free LimeSurvey® web application survey (**Appendix S1**) was carried out in 2015 among seven major Belgian veterinary laboratories to list the different assays that would be used in these three usual epidemiological scenarios. These laboratories were respectively the Federal Veterinary and Agrochemical Research Centre (CODA-CERVA), the regional laboratories (Association Régionale de Santé et d'Identification Animale (ARSIA) and Dierengezondheidszorg Vlaanderen (DGZ), the Public Health Institute (PHI), the Institute of Tropical Medicine of Antwerp and the laboratories of the Faculty of Veterinary Medicine of Liège University and Ghent University. Note that he CODA-CERVA and the Public Health Institute (PHI) were recently grouped in a unique institution, named Sciensano. In addition, the crisis emergency diagnosis and crisis management book that includes the assays and diagnostic capacities in crisis period was also consulted (data not shown).

Before submission of the questionnaire to the different laboratories, a pilot testing was performed with six experts (one responsible of a diagnostic laboratory at CODA-CERVA, a former responsible for diagnostic for small ruminants at DGZ, two senior epidemiologists trained in surveys and two junior epidemiologists of the CODA-CERVA).

All gathered information was captured in online knowledge maps using Cmap® software version 6.00.4, available on https://cmap.ihmc.us/. Knowledge maps were used because they have been demonstrated to be an effective mean of representing, visualizing, communicating knowledge between different stakeholders in the field of public health, improving theory development, acting as a sound basis for practical decision-making (Van Bon-Martens *et al*., 2014). For each disease, three arcs link the different scenarios and sub-scenario nodes using a specific colour code. Each node provides the tests that would be used in the Belgian laboratories or in the collaborating laboratories if external partnerships were in place (**Figure 1**).

2.3. Literature review on diagnostic assays

The aim of this review is to identify the main characteristics of the principal types of assays published but above all to guarantee they are used in the correct scenarios in the Belgian laboratories and to help finding diagnostic possibilities, if required.

The databases PubMed and Cab Abstract were consulted, considering the inclusion period starting from January 2000 and ending in February 2016, to get insight of the different published assays using the disease name as search term and using filters (see **Appendix S2**). The first selected papers are those for which the title was explicitly relevant (e.g. including the name of the disease and referring to the diagnosis or to a diagnostic test). After reading, within these selected papers, the major selection criteria were, in decreasing order of importance, the specific diagnosis of the pathogen (species, subspecies if possible) with a current technique, and the mention of the test sensitivity (Se) and/or specificity (Sp). A current technique can be defined as a technique still currently recommended in the literature and/or by the OIE (http://www.oie.int/en/) and the Centre for Food Safety and Public Health (http://www.cfsph.iastate.edu/).

The different diagnostic methods were subsequently classified according their capacity to detect directly or indirectly the pathogen/genome/antigen/antibodies.

2.4. Identification of diseases with major and minor diagnostic gaps in Belgium

Diseases are included in the group with "major diagnostic gaps" when no diagnostic tool validated for animal samples is available for the three scenarios in the Belgian laboratories. Diseases are included in the group with "minor diagnostic gaps" when some assays are not available or do not fit for purpose for at least one of the three scenarios. Diseases were not considered with diagnostic gaps in Belgium if official partnerships were already developed. Partnerships with other European laboratories guarantee rapid and reliable suspicion confirmation and support in diagnostic and in assays implementation during crisis by European reference laboratories.

Knowledge mapping was used to categorise diseases with major and/or minor diagnostic gaps (**Figure 2**). Then to assess the suitability of the Belgian assays, an expert elicitation was organized face-to-face with five team leaders of laboratories at CODA-CERVA. The purpose of this elicitation was to give a qualitative appraisal of the assay available regarding to the OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals as a reference. The qualitative appraisal for each scenario was the following: recommended method (+), suitable method (++) or may be used in some situations (+++).

In addition, to judge if the Belgian laboratories have or would have enough capacity to face a crisis, the quantity and the type of the different assays performed monthly by the national reference laboratory (NRL) and encoded in its Laboratory Information Management System (LIMS) were extracted from December 1999 to July 2015, for bluetongue (BT), foot-and-mouth disease (FMD) and avian influenza (AI). These diseases require an important capacity per day due to a high possibility to rapidly spread and to contaminate a large amount of naïve animals (O'Brien *et al*., 2017; *Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail*, 2012). During the previous crisis CODA-CERVA, as NRL, was the only laboratory allowed to test samples for FMD and avian influenza virus (AIV) whereas for bluetongue virus (BTV), next to CODA-CERVA also two public regional laboratories (ARSIA, DGZ) were involved. The number of assays performed

yearly, from 2006 to 2010 for bluetongue virus (BTV) at ARSIA and DGZ was also computed. These data were compared to the already published articles using the following search string performed in Cab abstracts "Foot and mouth OR bluetongue OR avian influenza AND laboratory capacit* OR epidemics".

2.5. Diseases with major and minor diagnostic gaps in Belgium: inventory of the diagnostic possibilities in some European laboratories

2.5.1. Diagnostic possibilities overview in Europe

To get an overview of the diagnostic possibilities (yes/no) for the 29 selected pathogens across Europe, the data available for 40 veterinary European laboratories inventoried in the World Animal Health Information System database (WAHIS) (https://www.oie.int/wahis_2/public/wahid.php/Countryinformation/Countrylaboratoris) were consulted in November 2019 for 23 of the 29 listed diseases. Note that Akabane (AKA), Aino disease (AD), Schmallenberg virus (SBV), Cache Valley virus (CVV), Porcine epidemic diarrhoea virus (PEDV) and Porcine deltacoronavirus (PDCoV) are currently not included in the OIE list (https://www.oie.int/en/animal-health-in-the-world/oie-listed-diseases-2019/).

These data were completed with the diagnosis possibilities in nine western European laboratories (NRLs and/or OIE reference centres) close to Belgium (2 in France, 1 in Germany, 1 in The Netherlands, 1 in Italy, 2 in Spain, 1 in Switzerland, 1 in United Kingdom). This information was obtained by mail and phone calls.

2.6. Diseases with minor diagnostic gaps in Belgium: inventory of the diagnostic possibility in some European laboratories

Among these nine laboratories, those with diagnostic possibilities for diseases with minor gaps in Belgium were re-contacted to complete the questionnaire for the diseases identified with minor diagnostic gaps in Belgium (**Figure 2**). The OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animal mentions that molecular and serological assays are the two main types of assays

used for crisis and for surveillance and freedom status establishment scenarios. Only these types of assays were considered as a possible solution to fill the gaps in Belgium.

2.7. Diseases with major diagnostic gaps in Belgium: inventory of the diagnostic possibility in some European laboratories

For the diseases with major gaps in Belgium, the query was extended to 12 OIE laboratories and 5 major public laboratories and university laboratories located in Western Europe (France: 3, Spain: 2, Italy: 1, United Kingdom: 3, The Netherlands: 3, Germany: 2, Denmark: 1, Sweden: 2). They were invited to fill the questionnaire for the three scenarios (**Figure 2**). If no diagnostic possibility exists, they were asked if diagnostic would be possible in other laboratories in their own country or in Europe (snowball approach).

2.8. Diseases with major diagnostic gaps in Europe: find diagnostic possibilities in laboratories outside Europe

If less than two assays were available per disease among all these European laboratories, other laboratories were contacted via the first author's address based on the literature review.

2.9. Inventory of the available commercial kits in Europe for the 29 selected diseases

The CODA-CERVA controls the quality of diagnostic reagents used for official animal disease control programs commissioned by the Federal Agency for the Safety of the Food Chain (FASFC). Commercial kits manufacturers/providers were selected within those who have controlled batches in the CODA-CERVA in 2013-2014. It was followed by a rapid internet search using the following algorithm: "disease name" AND "kit" OR "PCR" OR "ELISA" as keywords and Boolean operators. All major kits manufacturers/providers were included in the list. They were invited to complete the questionnaire (**Figure 2**). On the other hand, a cross check of the information received from firms was performed compared with those implemented on Discontools®. Discontools® is an online database with the objective to provide information related to the commercial diagnostic kits availability for 52 animal diseases and to identify where research is needed (https://www.discontools.eu/).

3. RESULTS

3.1. Inventory of the Belgian diagnostic possibilities and capacities for three epidemiological scenarios: input for knowledge mapping

The participation rate for the Belgian survey was 100%. Three laboratories were excluded because they did not propose routine testing (research only) or did not have diagnostic accreditation for animal samples.

A diagnostic possibility exists for 25/29 diseases. For confidential reasons, details concerning fees for the commercial kits and for diagnostic performed by European laboratories are not available for the public. Data were implemented in knowledge maps and available via this link: https://cmapspublic.ihmc.us/rid=1QZQ48NFK-2B2XY0S-30MB/page%20de%20garde%20EPIDIACAP%20(official).cmap.

3.2. Identification of diseases with major and minor diagnostic gaps in Belgium

Four diseases (Nipah virus (NiV), AD, CVV and AKA) are identified as diseases with major diagnostic gaps and three diseases (PDCoV, PEDV, haemorrhagic septicaemia (HS)) as diseases with minor diagnostic gaps (**Table 1**).

For PEDV and PDCoV, a validated real-time polymerase chain reaction (qPCR) as well as a serological assay for specific identification and discrimination of each pathogen is missing.

For HS samples are subcontracted to a human laboratory using laser desorption ionisation time-of-flight (Maldi-Tof). An alternative to Maldi-Tof should be implemented in a Belgian veterinary laboratory to face massive samples flow and when a serological assay is required.

Absence of virus isolation (VI) for Rift Valley fever is not considered as a lack because the disease is exotic in Europe and a biosafety-level-4 laboratory is required but genome that could be detected by qPCR as well as assays for antibody detections are available.

Table 1. Diagnostic possibilities for the 29 (re)-emerging diseases in Belgium (BE) (according to Bianchini *et al*., 2020), in Europe (EU), abroad Europe (INT) and kit availability by major manufacturers/providers (MP)

Diseases	Molecular diagnosis				Serological diagnosis			
	BE	EU	INT	MP	BE	EU	INT	MP
African horse sickness	0			1	0			0
African swine fever	0			0	0			0
Aino disease	1	1	0	1	1	1	0	1
Akabane	1	0		1	1	0		0
Avian influenza	0			0	0			0
Bluetongue	0			0	0			0
Cache Valley virus	1	1	0	1	1	1	0	1
Classical swine fever virus	0			0	0			0
Contagious bovine pleuropneumonia	0			1	0			0
Contagious caprine pleuropneumonia	0			1	0			0
Epizootic haemorrhagic disease	0			0	0			0
Equine encephalomyelitis (Eastern)	0			1	0			1
Equine encephalomyelitis (Venezuelan)	0			1	0			1
Equine encephalomyelitis (Western)	0			0	0			0
Foot and mouth disease	0			0	0			0
Haemorrhagic septicaemia	1*	1		0	1	0		1
Japanese encephalitis	0			1	0			1
Lumpy skin disease	0			0	0			0
Newcastle disease	0			0	0			0
Nipah virus encephalitis	1	1	0	1	1	1	0	1
Porcine epidemic diarrhoea	1	0		0	1	0		0
Porcine deltacoronavirus	1	1		0	1	1		1

Rift Valley Fever	0			1	0			0
Peste des petits ruminants	0			0	0			0
Schmallenberg	0			0	0			0
Sheep pox and goat pox	0			1	0			1
Swine vesicular disease	0			1	0			0
Vesicular stomatitis	0			1	0			1
West Nile encephalitis	0			1	0			1
Total	**7**	**4**	**0**	**15**	**7**	**4**	**0**	**11**

Legend: For respectively in Belgium (BE), in Europe (EU), outside Europe (INT) and kit availability by major manufacturers/providers (MP), the following code of colour is used: 0, the diagnostic possibility exists; 1: diseases with major or minor lack (no diagnostic possibility) and white, no lack or possible collaboration already found at BE or EU level. Total indicates the number of diseases for which laboratory diagnostic possibilities are still needed. *Samples subcontracted to a human laboratory.

Except for BTV, epizootic haemorrhagic disease (EHD) and vesicular stomatitis (VS) for which Belgium would use qPCR, results confirm that there is at least one OIE prescribed assay in Belgium, for each scenario, taking external partnerships into account.

Belgium is able to realise several thousand PCR, enzyme-linked immunosorbent assay **(**ELISA) and hemagglutination inhibition (HI) tests (**Figures 3 to 5**). During this episode, retrospective analysis in the LIMS, showed that CODA-CERVA can reach several thousand samples tested per month and saturation was not achieved. That means the whole process was efficient enough to enable performing the required analysis but also to ensure efficient logistics of the reagents and routing of the samples. The highest number of tests by competitive ELISA by the regional laboratories was 18,314 samples for ARSIA and 14,711 samples for DGZ in 2007. The mean for these two laboratories together is 21,578 ELISA tests per year. This flow could be adapted to other diseases. The flow preservation in time in crisis depends on the reactive availability and the time of delivery by commercial firms which varies from 2-3 to 21 days depending on their stocks.

3.3. Diseases with major and minor diagnostic gaps in Belgium: inventory of the diagnostic possibility in some European laboratories

3.3.1. Diagnostic possibilities overview in Europe

Data available on WAHIS for 40 European countries showed that 39 countries have diagnostic possibilities for Newcastle disease, 37 for AIV, 35 for FMD, CSF and BTV. Only 8 pathogens in the list could be diagnosed in more than 20 countries and there is no possibility for NiV (**Figure 6**).

In the 8/9 western European laboratories that answered for their diagnostic possibilities for the 29 diseases, all of them have specific tools for African horse sickness, African and classical swine fever, avian influenza, bluetongue, contagious bovine pleuropneumonia, Schmallenberg virus, swine vesicular disease, west Nile encephalitis but on the other hand, no possibility was found for CVV (**Table 2**). For AD, only the German laboratory and for NiV, the German (Henipavirus specific) and the Italian laboratories, have diagnostic possibilities. Note that based on the literature review, contact was also established with a laboratory in Denmark because it recently published an assay for haemorrhagic septicaemia that was considered as minor diagnostic lack in Belgium.

Table 2. Diagnostic possibilities in eight laboratories in Western Europe for the 29 diseases

Diseases	France (laboratory 1)	France (laboratory 2)	The Netherlands	Germany	Italy	Spain	Switzerland
African horse sickness							
African swine fever							
Aino disease							
Akabane					R		
Avian influenza							
Bluetongue							

Cache valley virus							
Classical swine fever virus							
Contagious bovine pleuropneumonia							
Contagious caprine pleuropneumonia							
Epizootic haemorrhagic disease							
Equine encephalomyelitis (Eastern)			ST				
Equine encephalomyelitis (Western)			ST				
Foot and mouth disease							
Haemorrhagic septicaemia				S			
Japanese encephalitis							R
Lumpy skin disease							
Newcastle disease							
Nipah virus encephalitis							
Peste des petits ruminants							
Porcine deltacoronavirus			ST	S	S	*	R*
Porcine epidemic diarrhoea						*	R*
Rift Valley Fever							
Schmallenberg							
Sheep pox and goat pox							
Swine vesicular disease							
Venezuelan Equine encephalomyelitis			ST				
Vesicular stomatitis			ST				
West Nile encephalitis		ST					

Legend: This table displays the diagnostic possibilities in 8 laboratories located in western European countries for the 29 selected diseases (Bianchini *et al*., 2020) based on mails and phone calls enquiries. Note that the two laboratories in Spain gave a common answer. Dark box: diagnostic possibility. White box: no diagnostic possibility. ST= subcontract abroad Europe. S=PCR soon available. R= for research purpose only. * No discrimination between porcine epidemic diarrhoea and porcine delta coronavirus.

3.3.2. Diseases with minor diagnostic gaps in Belgium: inventory of the diagnostic possibilities in some European laboratories

A specific qPCR for PEDV could be implemented from France, Italy and Germany but only the Netherlands proposed an optimized ELISA PEDV-specific based on the method published by Van Nieuwstadt and Zetstra (1991). Even if PDCoV can be detected with a Pan-coronavirus PCR, as implemented in Belgium, there were no specific, validated and commonly used molecular and serologic assays in Europe in 2015.

Concerning HS, Italy proposes a biochemical characterization, which is not really specific whereas Spain has developed different qPCR for serogrouping and gene virulence. Note that Denmark has developed a Multi Locus Sequence Typing (MLST) used in routine and as well as a qPCR (Petersen *et al*., 2014). MLST remains more costly and less throughput than qPCR. No serological assay was mentioned for the diagnostic of this bacterium.

3.3.3. Diseases with major diagnostic gaps in Belgium: inventory of the diagnostic possibilities in some European laboratories

Out of the 14/17 of European laboratories which completed the questionnaire, virus isolation, molecular and serologic assays were found for AKA in France and Germany. Only one diagnostic possibility was found in Europe for AD and CVV. For NiV, a specific PCR and a Henipah virus specific serological assays are available. As antibodies to Hendra virus and NiV cross-neutralize to a limited degree, ELISAs using Hendra virus or NiV antigen can be adequate to detect antibodies to both viruses (World Organisation for Animal Health, 2018b).

3.3.4. Diseases with major diagnostic gaps in Europe: find diagnostic possibilities in laboratories outside Europe

Based on the literature, diagnostic capabilities were found in the veterinary institutes in Republic of Korea and in Japan for AD (Akashi *et al*., 1999; Lee *et al*., 2015; Shin *et al*., 2009) and in the United-States for CVV (Wang *et al*., 2009). Histopathology is also used for the two viruses and is reliable for virus identification. For serology, both laboratories propose to implement virus neutralization test

(VNT), the gold standard serological screening in mammals, which has shown its usefulness in virus serogroups, species and subtypes identification (Blitvich *et al*., 2012; Weir, 2003). Several PCR have been developed in different formats (classical, nested, real-time, multiplex) and are used for genome detection in animal tissue, blood sample and insects (Lee *et al*., 2015; Shin *et al*., 2009; Wang *et al*., 2009).

3.4. Inventory of the available commercial kits in Europe for the 29 selected diseases

Considering the results from the eleven major manufacturers/providers (fifteen were contacted), 19 diseases could be covered with a commercial kit. In proportion, commercial assays available in Europe are PCR (30%) and antibodies ELISA (70%) which are not Differentiating Infected from Vaccinated Animals (DIVA), excepted for FMD. Three firms propose pen-side tests for African swine fever, AIV, BTV and PCRs for less equipped laboratories for AIV, CSF, FMD and Newcastle disease. Cross-checking with data available on Discontools®, no kit has been commercially developed for AD, CVV, Eastern/western/Venezuelan/Japanese encephalitis, NiV, Sheep and goat pox and VS. Caution should be taken when assessing the assay's sensitivity (Se) and specificity (Sp), especially when enssuring perfect sensitivity (Se.) and specificity (Sp). In this case, it should be assured that they have not been validated with a too restricted number of animals (<10) that could lead to assumed perfect tests (100% Se and 100% Sp) when all test results are positive or negative respectively while in reality this can not be the case given the confidence interval ranges for such small sample sizes. The first day post infection that the assay can be used is unknown or not evaluated for 65% of the kits and the last day is unknown or not evaluated for 74% of the kits.

3.5. Literature review on diagnostic assays

3.5.1. General assessments

A total of 840 articles were selected. In general, VI and VNT are considered as gold standard (MacLachlan and Dubovi, 2011) because of their high Se and Sp. However, because they require special equipment, stringent biosecurity measures, long laboratory preparation and a significant time before obtaining the results using these methods on a large number of samples in crisis is hampered. For genome detection, PCR was globally replaced by qPCR, which has in most cases an additional

number of advantages: higher Sp (due to the probe used), realtime availability of quantitative result (due to the measurement of fluorescence), automation (robot for automated deoxyribonucleic acid (DNA) extraction), and probability of contamination reduced (closed machine). However, the cost of technical equipment is still high especially for small labs or for low-income countries. In addition, a variant of these two techniques allows the simultaneous detection of several pathogens (multiplex). Advantage is the detection and the identification of virus from the same serogroup (i.e. AKA and AD) or virus with potential similar clinical signs (i.e. foetal malformation induced by, BTV, AKA, AD, CVV, SBV).

This, however, generally leads to a decrease Se. The major disadvantage of these molecular techniques is the inability to determine the viability of the identified agent, the susceptibility to contamination and the need to adapt to mutations in the genome. Sequencing uses amplicons of a first PCR and allows pathogen identification with great precision. The literature agrees that this technique will take an important place in the future for emerging or hard to cultivate pathogens' identification, etiological and phylogenetic research due to the price of the equipment, the need of trained personnel and the cost of use, this technique is still only used in well-equipped laboratories for specific confirmation and research. Loop mediated isothermal amplification (LAMP), another molecular technique, can be applied both in laboratory and in the field and has a high Se and Sp, often comparable to the qPCR. Other advantages are the speed at which the results are obtained (20 minutes) and the low cost per test. However, this technique has yet to be validated for routine use.

Regarding the ELISA for antibodies detection, numerous commercial tests, in-house and variants are available and discussed which made the comparison difficult, especially for the Se and the Sp. However, their major advantages are speed, ease of use, low cost and the ability to test a large number of samples simultaneously. Due to the time required between infection and onset of antibodies, this technique is generally applied for serological confirmation (e.g. confirmation of pathogen exposure in detection of disease, freedom status).

Data summarized for the 14 diseases of interest for Belgium present in Discontools® indicates that all diseases need improvements (i.e. assay development for Nipah, validation and harmonization, pen side test development, field validation, DIVA test development) (**Appendix S3**)

3.5.2. Specific assessments for diseases with major and minor diagnostic gaps

Less articles are available and fewer different types of assays have been developed for the diseases with major gaps compared to some other (i.e. FMD, BTV). For example, only 9 articles were considered as relevant for AD, 5 for CVV, 12 for AKA versus 32 for BTV, 60 for FMD and 134 for AIV. However, at least one molecular and serological test was published for each disease.

Differential diagnosis of PEDV and PDCoV (first reported in 2012) is essential to control viral diarrhoea but antibody cross-reactivity between porcine enteric coronaviruses remains a major concern (Gimenez-Lirola *et al*., 2017). However, some specific molecular (Ding *et al.*, 2020) and serological assays (Kimpston-Burkgren *et al*., 2020; Lu *et al*., 2020) allowing specific identification were recently published but required to be more largely implemented in all laboratories.

Some serological tests for HS detection have been published but are not commonly used for diagnostic purposes (World Organisation for Animal Health, 2018a), probability due to the few number of animals that survive and seroconvert.

3.5.3. Special assessments for vector borne diseases

If it was mentioned that qPCR was a key tool for genome detection, its use inherently depends on the presence of the genome in the matrix. Thus, for the viral vector borne diseases part of this study, the period of viremia is generally short and appears before the clinical signs, making early diagnosis by PCR or VI difficult. In this case, the serological methods are therefore preferred for the diagnostic. It also appears that serological confirmation of Flavivirus encephalitis remains difficult, in particular because of cross-reactions and absence of field test.

4. DISCUSSION

Diagnostic assays are routinely used in laboratories to demonstrate the presence or absence of current and/or past infections in different settings (i.e. early detection, characterisation of spread and freedom of disease). Because of growing incidence of new emerging pathogens with a high zoonotic potential

in wild and domestic animals, an effective laboratory service should be a priority before they become a threat to the public health and the animal health sectors. Europe already imposes to the member states to be efficient to face a crisis for some few listed animal diseases (European Regulation (EU) 2016/429) but it seems relevant to also be ready to face all potential emerging threats in different epidemiological scenarios. The actual epidemiologic situation seems under control for usual diseases, leading us to focus on the current diseases or the crisis of the moment rather than prioritizing our investments for emerging diseases. Due to this absence of urgent need and common guidelines at the European level, countries have adopted several strategies to prepare themselves to emerging diseases differently. Consequently, they have implemented different assays they consider to be the most suitable.

Countries should collaborate to develop a common list of diseases in the framework of a one health approach (wild life, animal health, human health) and each country should evaluate its own diagnostic capability to be sure to be efficient if the diseases appear. However, there is no published method applicable at the country level to overview the capabilities and over which tests should be implemented. Knowledge maps have been widely used and have proved to be valuable tools for description, classification and generalization of data (Ho *et al*., 2018) but to the authors' knowledge, it is the first time they are shown to be useful to provide an overview of the laboratory possibilities and capacities.

Several parameters should be considered to evaluate the capabilities: the type of gaps (major or minor) at national level, the possibility to find collaborations abroad, the kits availability from commercial firms, the diagnostic capacity, in the light of the scientific literature.

If diagnostic gaps are identified, without common and mandatory guidelines for all European laboratories, the OIE prescribed assays are considered as the reference. However, it is important to cross information from different scientific sources (e.g. the scientific literature, the OIE recommendations and Discontools®) to identify 'fake' gaps. As example, for African horse sickness, it is not relevant to detect the pathogen in blood during a crisis because of really short viremia duration. Not having any molecular assay in place cannot be considered as a gap. On the other side, OIE prescribed assays are sometimes not relevant for Europe and in agreement with the recent

scientific published articles. As example, there is a considerable variation in the diagnostic approach of BTV and EHD (Wilson *et al*., 2015). OIE does not promote PCR for BTV and EHD eradication and prescribes serology, probably because it has been intensively used and validated for surveillance and evaluation of mass vaccination (Katz *et al*., 2011). However, serology reveals the presence of antibodies either following past pathogen infection or transfer of maternal immunity and not necessarily active infection. On the other side, major limitations of PCR for these viruses are the possible detection of nucleic acids several weeks after the infection in animals no longer infectious as well as its susceptibility to contamination and its cost. In case of doubt, only VI could demonstrate the presence of the viable virus. In addition, laboratories must be able to adapt to the genetic diversity of the two viruses by selecting the most suitable probes for the new isolates. The experience acquired during the BTV-8 crisis in Belgium has shown the interest and complementarity of PCR and ELISA in different epidemiological scenarios (Vandenbussche *et al*., 2018; Welby *et al*., 2013). Even if genome could be detected earlier than antibodies in blood, PCR use costs 3 to 9 times more per sample than the ELISA and could explain why the last method is privileged when possible. Vesicular stomatitis is another example: the recommendation for eradication is to use serological tests (ELISA, VNT), which detect antibodies 5-8 days after infection. The competitive ELISA assay has become the serological test of choice for screening purposes in the United States during outbreaks of VS (European Food Safety Authority, 2012). A positive result indicates only previous exposure and requires, when interpreting the results, to also rely on the presence of clinical signs and to take into account the epidemiological situation. PCR can be performed 1-2 days before the appearance of lesions but is not particularly recommended by OIE because it cannot determine if the virus is infectious and because that tool is not routinely used for screening diagnostic cases for virus causing VS (World Organisation for Animal Health, 2015).

To find diagnostic possibilities, online knowledge maps could be used as a common modern media for communication and sharing information between laboratories. The One Health approach encourages collaborations between countries but the different medical disciplines and sectors have already developed their own country-specific diagnostic network with different levels of laboratories (i.e. WHO/OIE reference laboratories, NLR, regional laboratories, private laboratories). This stratification is sometime extremely complex to understand viewed from outside. Mapping and sharing diagnostic

flows online could permit to rapidly identify and connect the national, regional and international laboratories and entities to provide technical support and to establish or strengthening multilateral cooperation within a One Health laboratory network. They could also help to easier identify gaps in diagnostic as an alternative to Discontools ®, to enhance collaborations at international level, to share diagnostic experience and to harmonize diagnostic procedure based on the most recent scientific publication. However, maps need to be regularly updated to avoid contradiction, as it was observed when comparing data implemented in WAHIS (release date in 2013 but does not seem updated since) and the data gathered directly by contacting the laboratories. As example of possible collaboration, this article underlined disparities in sensitivity and specificity and in the window post-infection/inoculation during which the assays can be used. Explanation could be found, independently of the assay, due to the lack of investment to completely evaluate rare disease, the different study protocols in the different countries, including a variable number of animals, species, breeds, ages, inoculation method, strain and dose injected. Test interpretation, especially the determined cut-off, is subject to modifications. *In vivo* evaluations and meta-analysis would be useful to fill these gaps, which could be achieved in a cheaper way within a European consortium.

It is also impractical and excessively costly for most countries to maintain a national veterinary diagnostic laboratory that has full capabilities for confirmatory diagnosis of all emergency diseases. Another example of collaboration could be to enhance materials and samples dedicated for the different epidemiological scenarios, enhance different cooperation between countries to reduce the cost of diagnostic kits development, validation and renewal. To guarantee sufficient resources in times of reduced financial attribution, a European kit stock could be elaborated with design reference laboratories in charge to accredit assays, to ensure a rapid help if requested, to better cover the emerging diseases and to supply sufficient harmonization of international guidelines and standards for laboratories.

It is challenging to evaluate the required diagnostic capacity because this information is rarely published and countries do not make it public. This could be evaluated by combining crisis spread modelling, literature review (published data based on previous crisis), and by experts elicitation. For example, in 2006, Belgium faced a BTV crisis and showed its practical capability to deal with the

number of samples submitted. For FMD, based on Bouma (2003) (Bouma *et al*., 2003), Belgium seems to have enough capacity to face a crisis. This article clearly mentions the capacity developed during the FMD crisis in the Netherlands in 2001. During the month the crisis occurred, 78 samples (standard deviation 61) samples were daily analysed by antigen ELISA and, when vaccination began, 50 000 samples weekly by DIVA serology. Belgium could develop much more diagnostic capacities even if animal density is lower compared to the Dutch region where FMD outbreak happened.

For the other diseases still exotic to Belgium but for which diagnostic possibilities are available, the NRL prepared itself to face an emergence. To do so, a crisis manual was elaborated in which for each disease and for each test available important information is listed such as the maximum daily capacities per person and in total, the collaborations with other laboratories in Belgium and abroad, the time required before results will be available. This manual is regularly updated and validated by experts working for the FASFC.

To make comparisons between countries possible, it is more relevant to base assessments on daily diagnostic capacity needed by animal density than on only on a diagnostic capacity achievable per day.

This study highlighted the potential usefulness of knowledge mapping in identifying diagnostic gaps. However, it was limited to Belgium and did not include a real systematic review of literature. A proposal for future research would be to have the tool shared on the web in order to involve other countries and to combine it with systematic review.

In conclusion, this study underlined the necessity for each European country to evaluate its diagnostic capabilities for a common list of (re-)emerging diseases in order to be prepared. Knowledge mapping could be a useful tool not only to visually identify major and minor lack(s) in diagnostic in frequent epidemiological scenarios but also for sharing knowledge and enhance collaborations with other countries.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ETHICAL APPROVAL

Due to the nature of the study and the low risk exposure of the participants, formal approval from an Ethics Committee was not a requirement at the time of the study.

ORCID

Claude Saegerman https://orcid.org/0000-0001-9087-7436

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Figure and appendix captions

Figure 1. Knowledge mapping structure used to highlight diagnostic gaps in three epidemiological scenarios (early detection, disease spread, and freedom)

Figure 2. Decision tree to categorise diseases with major and minor diagnostic gaps in Belgium

Legend: LIMS, laboratory information management system; AIV, avian influenza virus; FMD, foot-and-mouth disease; BTV, Bluetongue virus; OIE, World Organisation for Animal Health.

Figure 3. Number of tests for foot and mouth disease per month realized at the CODA-CERVA from December 1999 to December 2001

Figure 4. Number of tests for bluetongue disease per month realized at the CODA-CERVA from April 2006 to April 2015

Figure 5a. Number of test for avian influenza tests per month realized at the CODA-CERVA from January 2000 and March 2015

Figure 5b. Number of avian influenza PCR monthly realized at the CODA-CERVA from January 2003 and July 2015

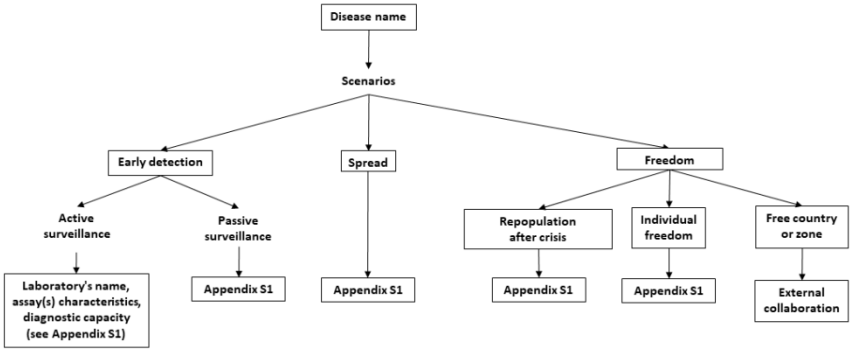
Figure 6. Number of European countries with diagnostic possibility per pathogen, based on the World Animal Health Information System (WAHIS) database

Legend: WAHIS, World Animal Health Information System. European countries encoded in the WAHIS database also includes non-European union member states.

Appendix S1. Items considered in the questionnaire sent to the laboratories

Appendix S2. Filters used for the literature review in PubMed and Cab Abstract

Appendix S3. Identification of diagnostic requirements for 14 diseases of interest for Belgium present in Discontools®



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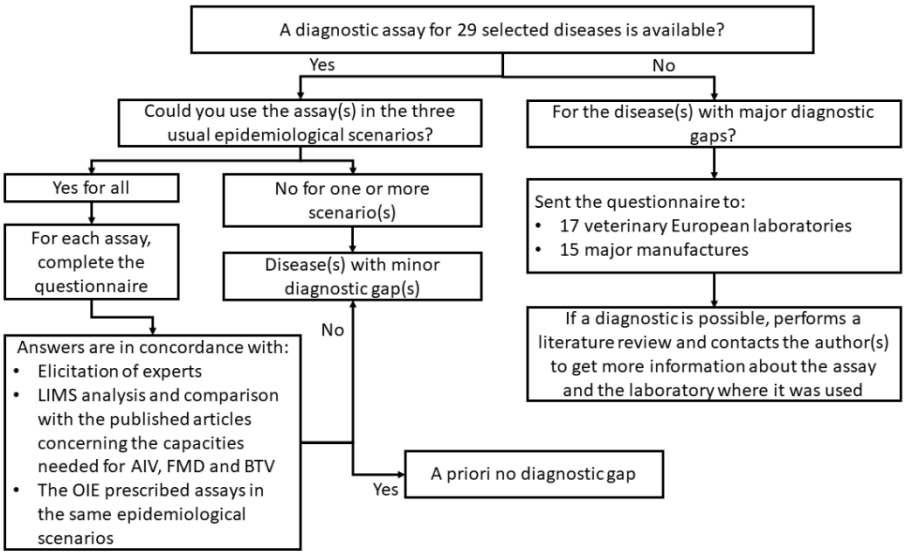

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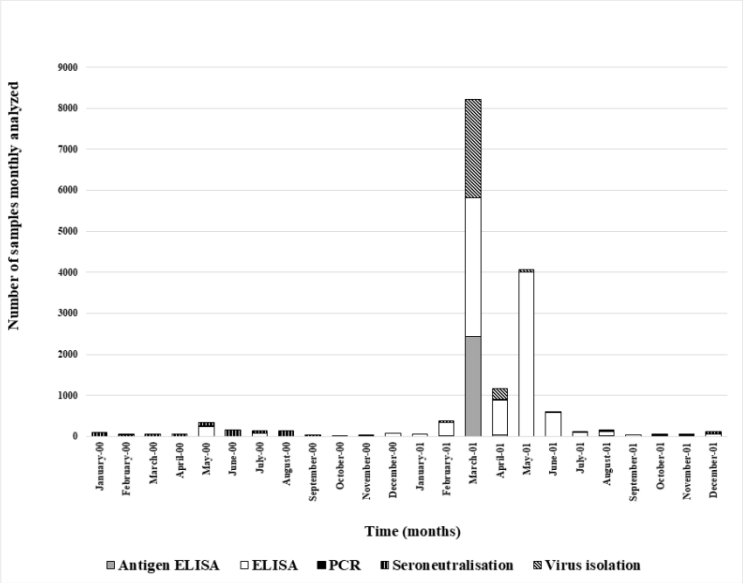

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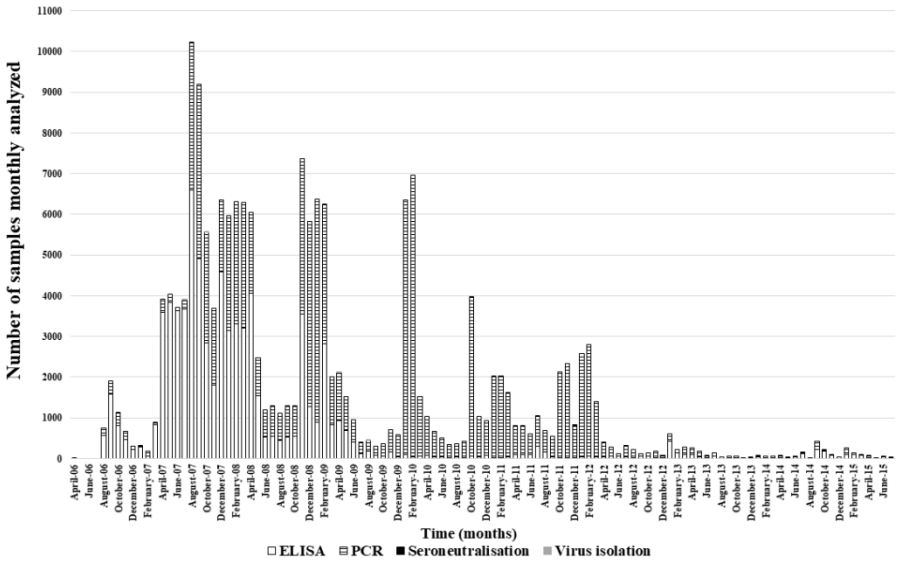

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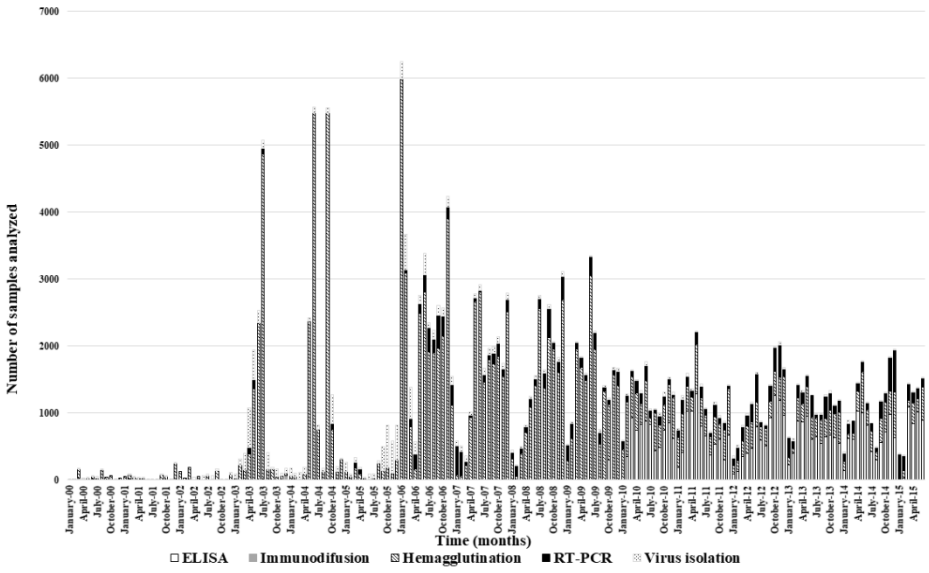

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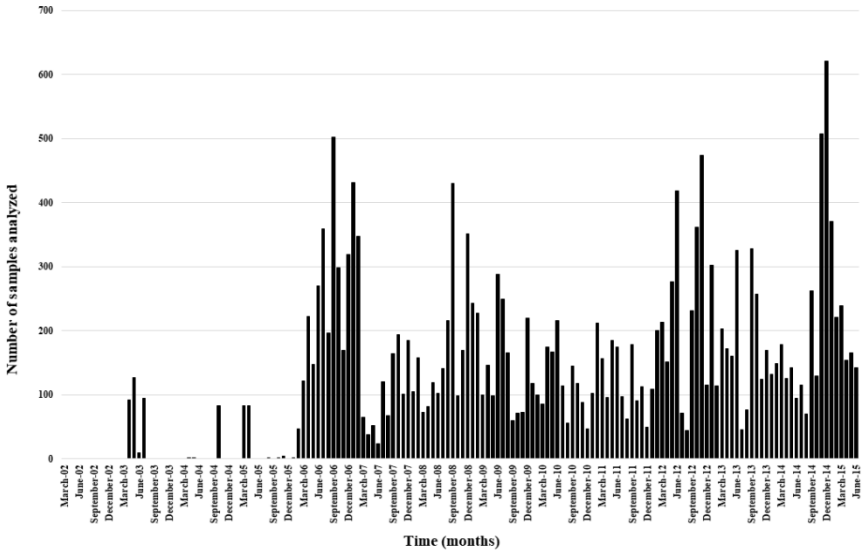

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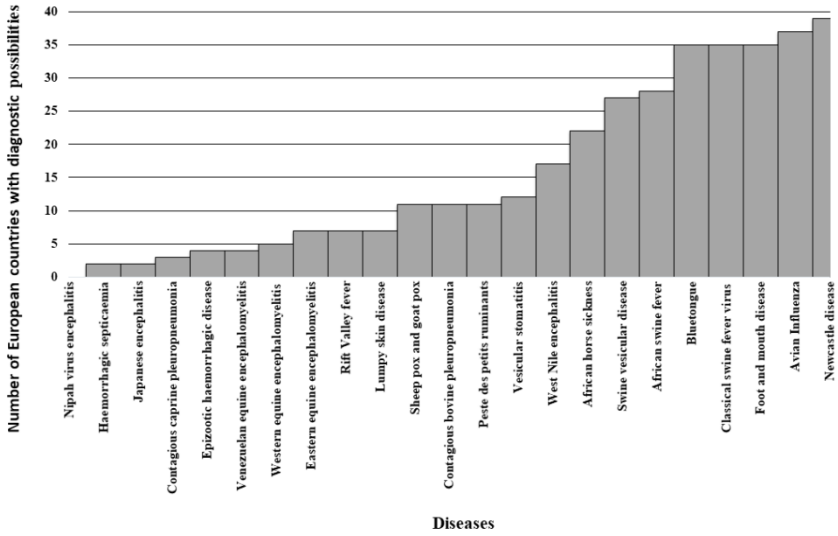

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