European survey on laboratory preparedness, response and diagnostic capacity for Crimean-Congo haemorrhagic fever, 2012

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Crimean-Congo haemorrhagic fever (CCHF) is an infectious viral disease that has (re-)emerged in the last decade in south-eastern Europe, and there is a risk for further geographical expansion to western Europe. Here we report the results of a survey covering 28 countries, conducted in 2012 among the member laboratories of the European Network for Diagnostics of 'Imported' Viral Diseases (ENIVD) to assess laboratory preparedness and response capacities for CCHF. The answers of 31 laboratories of the European region regarding CCHF case definition, training necessity, biosafety, quality assurance and diagnostic tests are presented. In addition, we identified the lack of a Regional Reference Expert Laboratory in or near endemic areas. Moreover, a comprehensive review of the biosafety level suitable to the reality of endemic areas is needed. These issues are challenges that should be addressed by European public health authorities. However, all respondent laboratories have suitable diagnostic capacities for the current situation.

Introduction

Crimean-Congo haemorrhagic fever (CCHF) is a zoonotic viral disease caused by the tick-borne CCHF virus (CCHFV), which is classified into the genus *Nairovirus* within the *Bunyaviridae* family. In humans, the disease is highly pathogenic and life-threatening as it can cause severe illness with prominent haemorrhages reaching case fatality rates of up to 50%. In nature, CCHFV usually circulates between asymptomatic animals and ticks in an enzootic cycle. Humans may become infected through the bite of a tick, mainly of the *Hyalomma* genus, through direct contact with blood or tissues from viraemic livestock or through direct contact with the blood or secretions of a viraemic patient [1]. Thus, risk groups include individuals with outdoor activities, mainly those who have occupational contact with animals, as well as healthcare workers in hospital settings (nosocomial hazard). Because of the potential for epidemics and nosocomial outbreaks, high fatality ratio, limitations for treatment and the lack of safe vaccine, CCHF is a disease listed for immediate notification to public health authorities as it constitutes a major threat to public health. Therefore, CCHFV is considered a high-risk pathogenic organism and classified as a biosafety level (BSL) 4 containment agent.

The disease is endemic in wide areas of Africa, the Middle East, central and south-western Asia and the south-eastern European region. More particularly, some Balkan countries (e.g. Albania, Bulgaria, Greece and Kosovo under UN Security Council Resolution 1244) are endemic zones for CCHF [2]. During the last decade, CCHF re-emerged in Albania, Greece, Kosovo under UN Security Council Resolution 1244 and countries bordering the Black sea: Georgia, south-western Russia Turkey, and Ukraine. In Greece, the detection of the non-pathogenic strain AP92 in ticks in 1975 was followed by the notification of the first human CCHF case in June 2008 [3]. However, the vast majority of CCHF cases have been recorded in Turkey (since 2002) and the south-western regions of Russia (since 1999), with expanding outbreaks and increasing numbers of associated fatalities [2]. In northern and south-western Europe, no human cases have been reported except for imported ones in France [4], Germany [5] and the United Kingdom [6]. Limited serological evidence in humans has been reported in parts of Hungary and Portugal [7,8].

In Europe, the tick vector most commonly associated with CCHFV is *Hyalomma marginatum*, which is present in southern Europe and has sporadically been detected in southern Germany, the Netherlands and the United Kingdom following expansion of its geographical range associated with movement of migrant breeding birds [9-12]. The spreading of the vector represents a risk factor for introduction of the virus from endemic to unaffected areas of Europe, increasing the occurrence of CCHF [13]. However, virological evidence has never been addressed in western Europe until 2010, when a study conducted in Spain detected for the first time CCHFV in populations of *H. lusitanicum* collected from indigenous deer [14]. Moreover, the recent discovery of antibodies against CCHFV in livestock in Romania, with prevalence values similar to those observed in other regions where the disease is endemic, suggests an extension of the circulation zone of CCHFV in Europe [15].

In 2008, after the first case in Greece was detected, the European Centre for Disease Prevention and Control (ECDC) organised an expert consultation on CCHF to identify preparedness interventions in Europe [13]. In 2011, under the initiative of the European Network for Diagnostics of 'Imported' Viral Diseases (ENIVD; www.enivd.org), a multicenter study of CCHF diagnostic tests and an external quality assessment

Table 1

ENIVD survey on Crimean-Congo haemorrhagic fever, responding laboratories, by country, 2012 (n=31)

Participating countries	Participating laboratories	Acts as NRL	WHOCC
Austria	Medical University of Vienna, Vienna	No	No
Belgium	Institute of Tropical Medicine, Antwerpen	Yes	No
Bulgaria	National Centre for Infectious and Parasitic Diseases, Sofia	Yes	No
Croatia	University Hospital for Infectious Diseases, Zagreb	No	No
Czech Republic	Institute of Public Health, Ostrava	No	No
Estonia	National Institute for Health Development/Health Board, Tallinn	No	No
Former Yugoslav Republic of Macedonia	Institute of Health Protection of the FYROM	No	No
France	1. Institut Pasteur, Lyon	Yes	Yes
	2. Aix-Marseille University and AP-HM Public Hospitals, Marseille	No	No
Germany	1. Bernhard-Nocht Institut, Hamburg	Yes	Yes
	2. Institut für Mikrobiologie der Bundeswehr, Munich	No	No
Greece	Aristotle University, Thessaloniki	Yes	No (discontinued since 20/ Oct/2008)[a]
Italy	National Institute for Infectious Diseases "L.Spallanzani", Rome	Yes	Yes
Kosovo under UN Security Council Resolution 1244	National Institute of Public Health of Kosovo, Pristina	Yes	No
Latvia	Infectology Center of Latvia, Riga	Yes	No
Lithuania	National Public Health Surveillance Laboratory, Vilnius	Yes	No
Malta	Mater Dei Hospital, Valletta	No	No
The Netherlands	Erasmus University Hospital, Rotterdam	Yes	Yes
Norway	Norwegian Institute of Public Health, Oslo	Yes	No
Portugal	National Institute of Health, Águas de Moura	Yes	No
Romania	National Institute of Public Health, Bucharest	Yes	No
Russia	Central Research Institute of Epidemiology, Moscow	Yes	No
Serbia	Torlak Institute of Virology, Belgrade	Yes	No
Slovakia	Institute of Virology, Slovak Academy of Sciences, Bratislava	No	No
Slovenia	University of Ljubljana, Ljubljana	Yes	No (discontinued since 1/ Sep/2008)[a]
Spain	Instituto de Salud Carlos III, Madrid	Yes	No
Sweden	Swedish Institute for Infectious Disease Control, Karolinska Institute Stockholm, Solna	Yes	No
Switzerland	University Hospitals of Geneva, Geneva	Yes	No
Turkey	Refik Saydam Hygiene Institute, Ankara	Yes	No
United Kingdom	1. Public Health England, Colindale	No	No
	2. Public Health England, Porton Down	Yes	Yes

ENIVD: European Network for Diagnostics of 'Imported' Viral Diseases; NRL: National Reference Laboratory; WHOCC: World Health Organization Collaborating Center (http://apps.who.int/whocc/) for Viral Haemorrhagic Fevers from the EURO region.

[a] Discontinued means that the institution is no longer a WHOCC.

(EQA) for CCHF molecular diagnosis were carried out to monitor and compare the performance of the different techniques applied for diagnosis of CCHF [16,17]. The current situation with continous high transmission in Turkey and south-western Russia, new imported cases in the European Union (EU), detection of the virus for the first time in the western Mediterranean region, and new evidence of seroprevalence in animals, make necessary a new assessment on preparedness and laboratory capacities for CCHF in the European region. Here, we describe the results of a questionnaire survey conducted in 2012 to assess the laboratory preparedness and response capacities for CCHF diagnosis in the European region.

Methods

To gather information on CCHF diagnostics, preparedness and response capacities in Europe, a questionnaire was developed and sent electronically in January 2012 to laboratory contact points in the ENIVD database, covering 28 Member States of the EU as well as nine non-EU countries, Russia, Norway, Switzerland, Bosnia and Herzegovina, Serbia, Kosovo under UN Security Council Resolution 1244, Albania, the Former Yugoslavia Republic of Macedonia and Turkey. All completed questionnaires were received by April 2012. The first part of the questionnaire assessed preparedness and response capacities, while the second part was designed to collect information on diagnostic capacities and quality assurance. Questions on the following topics were included in the questionnaire: CCHF case definition, training necessity, biosafety assurance, diagnostic tests and quality assurance. The list of respondents is shown in Table 1. Respondents were National Reference Laboratories (NRL) for Arbovirus and Viral Haemorrhagic Fever (VHF) and/or World Health Organization Collaborating Centers (WHOCC). An NRL was defined as a laboratory involved in reception/management of suspected samples of CCHF, either for diagnostic and reference activities or for shipment abroad in case of lack diagnostic capacity

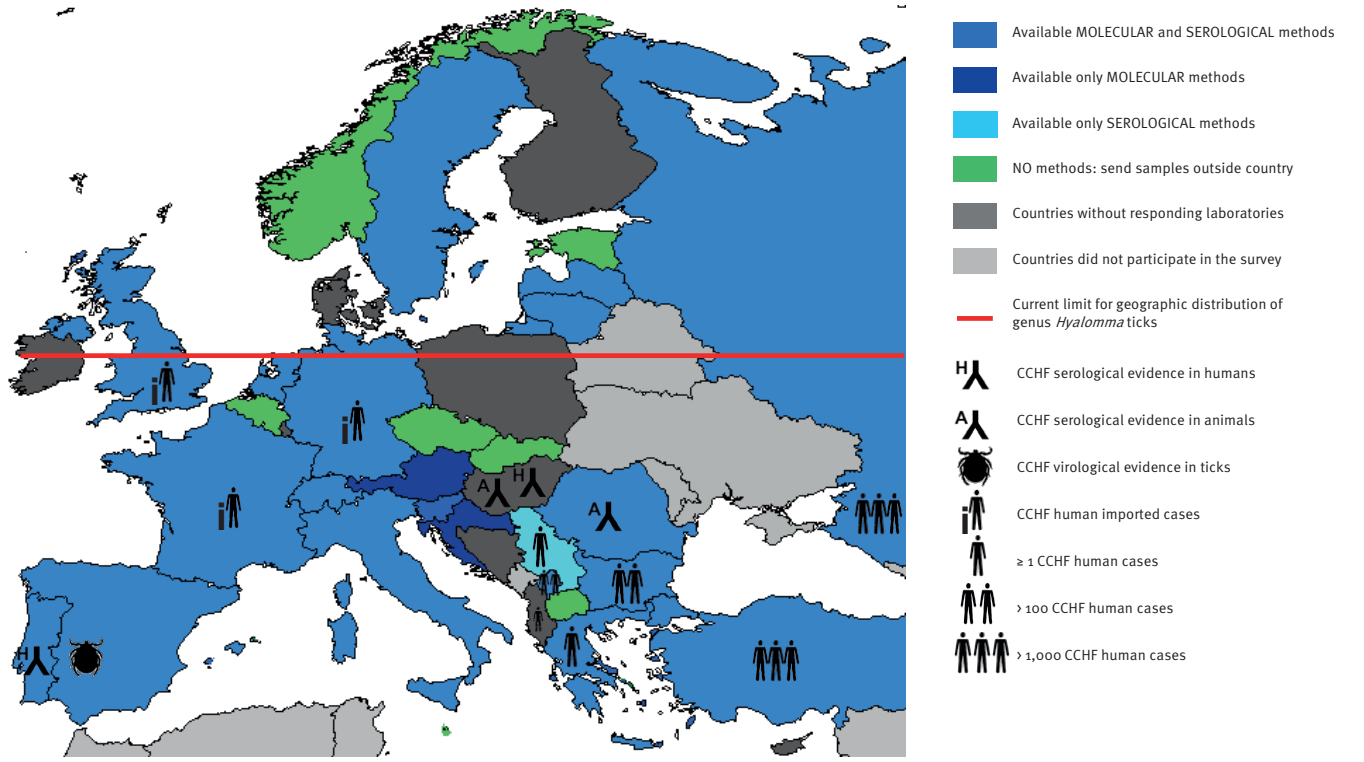
Results

Participation

Sixty-eight laboratories from 37 countries (28 EU Members States and nine countries outside the EU), were contacted for this survey. Thirty-one laboratories from 28 countries returned their answer, except Albania, Bosnia and Herzegovina, Cyprus, Denmark,

Figure 1

Diagnostic capacities and occurrence of Crimean-Congo haemorrhagic fever in Europe since 2000



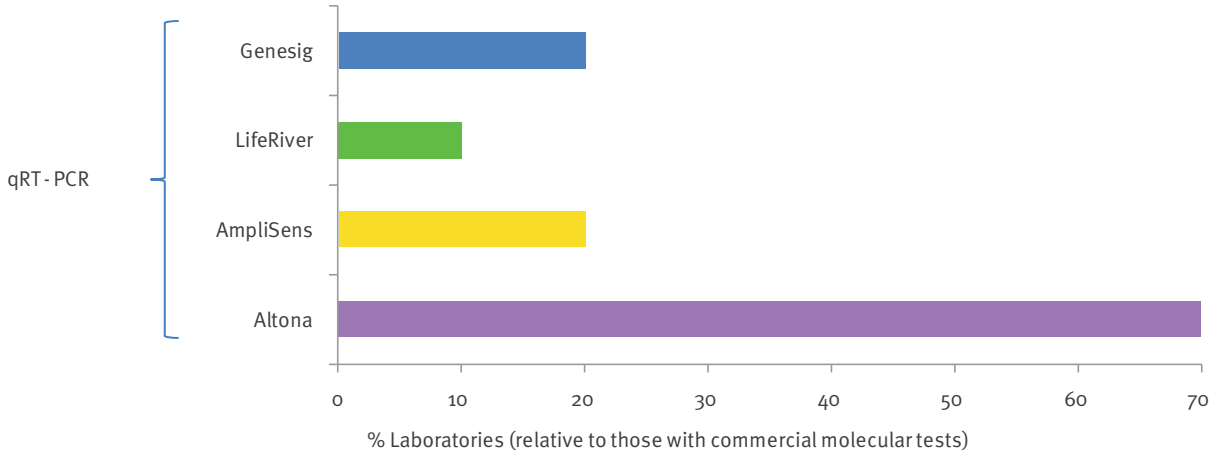
CCHF: Crimean-Congo haemorrhagic fever; ECDC: European Centre for Disease Prevention and Control; WHO: World Health Organization.

Colour code indicates diagnostic capacities as assessed in the present survey. Human silhouettes indicate occurrence of CCHF in humans according to the WHO database (http://data.euro.who.int/cisid), the ECDC consultation [13] and the Public Health England database (http://www.hpa.org.uk/Topics/InfectiousDiseases/InfectionsAZ/CCHF/EpidemiologicalData/cchfoutbreaks/). Tick silhouettes indicate virological evidence of CCHF in ticks in those countries where no human cases have been reported. Antibody silhouettes indicate serological evidence of CCHF in humans or animals in countries where no human cases have been reported.

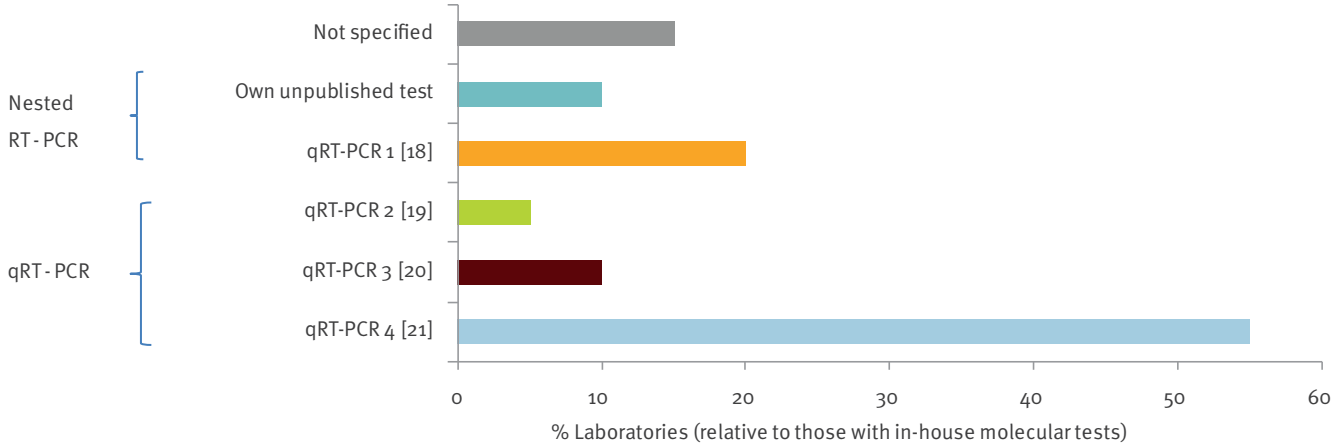
Figure 2

Application of Crimean-Congo haemorrhagic fever molecular diagnostic methods, ENIVD survey, 2012 (n=23 laboratories)

A. Percentage of countries using commercial molecular tests



B. Percentage of countries using in-house molecular tests



- Kosovo under UN Security Council Resolution 1244 , Romania
- Latvia
- Lithuania, Russia
- Germany (Hamburg, Munich), Greece, Kosovo under UN Security Council Resolution 1244, Portugal, Slovenia, Turkey
- France Lyon, Germany Munich, Romania
- The Netherlands, Russia, Spain
- Bulgaria, Croatia, Greece, Slovenia
- Slovenia
- Greece, Italy
- Austria, France Marseille, Germany Hamburg, Greece, Kosovo under UN Security Council Resolution 1244Sweden, United Kingdom Colindale, United Kingdom Porton Down

ENIVD: European Network for Diagnostics of 'Imported' Viral Diseases; qRT-PCR: quantitative real-time reverse transcription polymerase chain reaction; RT-PCR: reverse transcription polymerase chain reaction.

Finland, Hungary, Ireland, Luxembourg and Poland, corresponding to a participation of 76% of the countries and 45% of the laboratories. Of all laboratories that participated in the survey, 21 acted as a NRL for VHF and five act as a WHOCC.

Preparedness and response

All respondent laboratories declared that CCHF was a notifiable disease in their countries and that they followed the generic case definition for VHFs, while six countries (Bulgaria, Greece, Germany, Turkey, Russia and Spain) had their own case definition for CCHF (Table 2).

Most laboratories (25/31) stated that they had trained staff authorised to handle CCHF samples and that there was trained staff in their countries skilled in assessing VHF cases/outbreaks; 19 laboratories emphasised a need for further training, not only for laboratory workers, but also for medical and nursing staff. Half of the 24 laboratories with CCHF diagnostic capacity stated their availability to offer training services for CCHF diagnosis to other laboratories in and outside their countries.

Of all responding laboratories, 20 had standardised procedures for specimen collection and storage of CCHF infected material, and 25 for processing and shipping suspected CCHF specimens for confirmation diagnosis in other laboratories.

Diagnostic capacities

Of the 31 laboratories that participated in the survey, 24 declared to have set up diagnostic capacities to detect CCHF infection. The remaining seven laboratories in countries where CCHF diagnostic capacities has not yet been established, declared that they were sending samples to reference laboratories or WHOCCs outside their countries (Figure 1).

Among the 24 laboratories with diagnostic capacities, all except the laboratory in Serbia had CCHF molecular tests based on either quantitative real-time reverse transcription polymerase chain reaction (qRT-PCR) or nested RT-PCR. Information on the type of commercial or in-house protocol used was requested from the participants. Among the 23 laboratories which performed CCHF molecular diagnosis, 20 used an in-house method, 11 used commercial assays and eight combined both in-house and commercial approaches (Figure 2).

The serological diagnosis of CCHFV infection is based on the detection of specific IgM and IgG antibodies against recombinant nucleoprotein as the predominant available antigen, either in an enzyme-linked immunosorbent assay (ELISA) or in an indirect immunofluorescence assay (IFA). Most laboratories (22/24) with diagnostic capacities had available at least one serological technique, ELISA or IFA. Respondents were also asked about the availability of specific in-house or commercial (Vector-Best, Novosibirsk, Russia) ELISA tests for CCHF as well as in-house or commercial (EuroImmun, Luebeck, Germany) IFA (Table 2).

Eleven of 21 countries declared doing research to improve in-house molecular methods, and six of the 21 declared investigating also new serological in-house methods.

Regarding quality assurance, this survey revealed that 19 of the 23 laboratories with molecular diagnostic methods participated in the EQA on CCHF molecular diagnosis organised by ENIVD in 2011 [16], while only four participated in the exercise organised by the Quality Assurance exercise and Networking on the Detection of Highly Infectious Pathogens (QUANDHIP) project (www.quandhip.info) (Table 3).

Biosafety

The 24 laboratories with diagnostic capacities informed about the inactivation process before handling specimens for diagnostic purpose. Among them, six laboratories inactivated specimens under BSL4, 12 in BSL3 and five in BSL2 conditions (Figure 3). Of 11 laboratories performing viral isolation and propagation, six did so in BSL4 facilities and five in lower-grade BSL facilities.

Discussion

This survey has been carried out in 28 countries of the European region, including 10 countries where human cases are frequently or sporadically reported, or where there has been evidence of CCHFV circulation in animals or ticks. The presence of potential CCHFV vectors in other European countries may extend the current geographical distribution of the disease. In addition, imported cases in travellers have been reported in the EU. Hence, early recognition of the suspected CCHF cases is critical, in order to initiate the proper treatment of the patient and to apply control measures for containment of the disease. Some authors argue that Europe needs to implement a harmonised case definition for CCHF in order to enhance notifications and to estimate the diseases burden and epidemiological trends in various areas and countries [2]. The survey revealed that all responding countries used the generic case definition of VHFs to identify and notify CCHF cases. However, this survey has some limitations since not all responding countries clearly specified the source and reference of the generic or specific case definitions.

Networking and training are key factors in ensuring a rapid and effective response to CCHF. The survey revealed that the majority of countries belong to at least one network apart from ENIVD that could assure support, management, training in the diagnosis of CCHF cases, expert consultation, exchange of experiences and protocols, and scientific support if needed. Considering that some respondents did not have procedures in place for specimen collection, processing or

Table 2

Application of Crimean-Congo haemorrhagic fever serological diagnostic methods, ENIVD survey, 2012 (n=22 respondents)

Serological diagnostic method	Countries	Proportion of countries (relative to those with CCHF serological tests)
Commercial assay	Bulgaria, Latvia, Lithuania, the Netherlands, Portugal, Romania, Spain	37%
In-house assay	France, Serbia, Sweden, Switzerland, United Kingdom	26%
Commercial and in-house assay	Germany, Greece, Kosovo under UN Security Council Resolution 1244, Italy, Russia, Slovenia, Turkey	42%

CCHF: Crimean-Congo haemorrhagic fever; ELISA: enzyme-linked immunosorbent assay; ENIVD: European Network for Diagnostics of 'Imported' Viral Diseases; IFA: indirect immunofluorescence assay..

transporting, the networks could also play a key role in closing this gap. The networks could also foster training via organising international workshops on CCHF diagnosis and biosafety.

Laboratory techniques are the cornerstone of CCHF diagnosis, essential for effective surveillance, management of individual patients and outbreak prevention. In 2008, the multidisciplinary consultation of CCHF experts organised by ECDC showed that according to ENIVD, 15 of 27 countries performed CCHF diagnostics [13].The current survey launched in 2012 indicated an increase to 21 of 28 countries performing CCHF diagnostics. Our results show a strong increase in the diagnostic capacity for CCHF from 2008 to the present, possibly due to the nomination of CCHF as a priority disease for the EU. However, as shown in Table 1, two WHOCC next to endemic areas (Greece and Slovenia), lost their status as reference centres for VHF. This issue has to be taken in consideration when a new reference centre in Europe will be designated in the future.

Currently, the routine laboratory diagnosis of CCHF is based mainly on the detection of the viral genome and specific IgM and IgG. Most surveyed laboratories with diagnostic capacities (21/24) followed international recommendations of combining molecular and serological methods for CCHF diagnosis [1,28]. This shows that most of the surveyed laboratories have essential diagnostic tools for CCHF diagnosis in place.

Molecular assays offer a rapid, sensitive and specific diagnosis of CCHF during the viraemic phase of infection up to day 16 of illness [29]. The vast majority of surveyed countries (20/21) have molecular tests available, and most of them participated in CCHF EQAs. It is highly recommended that not only endemic countries, but also neighbouring countries that lack the capacity for molecular assays try to implement them.

Of the existing molecular methods for CCHF diagnosis, the majority of respondents (18/20) used a qRT-PCR, combined or not with nested PCR, while the remaining two countries used a nested RT-PCR only. Moreover, in a recent molecular EQA, it is reported that nested RT-PCR performs considerably less well compared with qRT-PCRs [16]. Therefore, it is recommended that countries performing only nested RT-PCR implement capacities for a quantitative assay because qRT-PCRs offer advantages when over nested RT-PCR such as lower contamination rate, higher sensitivity and specificity, and better time-effectiveness. A factor that may limit the use of molecular diagnostic methods is the fact that sensitivity may be affected by the high diversity of CCHF genomes. For instance, it has been found that sensitivity of molecular methods was associated with the patients' country of origin [17]. A combination of commercial and in-house RT-PCR assays will probably ensure the detection of CCHFV strains despite their diversity. However, the survey reveals that 20 of 23 laboratories use in-house RT-PCR but only eight combine it with a commercial test.

Although serological methods may cover a broader spectrum of strains due to cross-reactivity, attention must be also paid to antigenic variation among CCHFV strains which may affect their sensitivity. However, combinations of ELISA and IFA, commercial or in-house, may increase the sensitivity of detection. A recent evaluation of two commercial kits (VectorBest ELISAs and Euroinmune IFA, both for IgM and IgG) revealed that efficient and well characterised serological assays and protocols are available for CCHF diagnosis [17]. Our survey reveals that all countries using the commercial ELISA also had available commercial IFAs and that half of them combined them with an in-house ELISAs that may compensate a potential lower sensitivity caused by antigenic diversity. We advise that each country assure that their methods are optimised for strains circulating in their area, or that they use an adapted method for CCHFV genotypes circulating in their country.

In addition, to assure that diagnostic methods perform with optimal accuracy, an increased effort is needed to establish EQA studies on a regular basis. In 2011, an international EQA for the molecular detection of CCHF was launched [16]. The majority of countries with areas endemic for or at risk of CCHF surveyed in our study

Table 3

Laboratory preparedness and response capacities for Crimean-Congo haemorrhagic fever diagnosis in the European region, ENIVD survey, 2012 (n=28 countries)

Countries	Preparedness and response					Diagnostic methods	
	Case definition		Networks	EQA		Diagnostic techniques	BSL
	Generic VHFf	Specific CCHF		ENIVD	QUANDHIP		
Austria	Yes	No	ENIVD	Yes	No	PCR	BSL2+
Belgium	NA	No	ENIVD	No	No	Referral	
Bulgaria	NA	Yes[a]	ENIVD, EpiSouth, CCH-FEVER	Yes	No	PCR, ELISA, IFA, VI	BSL2
Croatia	Yes	Yes[a]	ENIVD	No	No	PCR	BSL3
Czech Republic	NA	No	ENIVD	No	No	Referral	
Estonia	Yes	No	ENIVD	No	No	Referral	
Former Yugoslav Republic of Macedonia	NA	Yes[b]	ENIVD, EpiSouth	No	No	Referral	
France	NA	No	ENIVD, EpiSouth, Euronet-P4	Yes (Lyon)	No	PCR, ELISA, VI	BSL4
Germany	NA	Yes	ENIVD, Euronet-P4	Yes (Hamburg)	Yes	PCR, ELISA, IFA, VI	BSL4
Greece	NA	Yes[c]	ENIVD, EpiSouth, Arbo-Zoo-net, CCH-FEVER	Yes	No	PCR, ELISA, IFA, VI	BSL3
Italy	NA	No	ENIVD, EpiSouth, Euronet-P4	Yes	Yes	PCR, IFA, VI	BSL4
Kosovo under UN Security Council Resolution 1244	NA	Yes	ENIVD, EpiSouth	No	No	PCR, ELISA, IFA	BSL2
Latvia	NA	Yes	ENIVD	Yes	No	PCR, IFA	BSL3
Lithuania	NA	No	ENIVD	No	No	PCR, IFA, VI	BSL3
Malta	Yes (ECDC)	No	ENIVD, EpiSouth	No	No	Referral	
The Netherlands	Yes	No	ENIVD	Yes	No	PCR, IFA	BSL3
Norway	Yes	No	ENIVD	No	No	Referral	
Portugal	NA	No	ENIVD	Yes	No	PCR, ELISA, IFA	BSL3
Romania	Yes	No	ENIVD, EpiSouth	Yes	No	PCR, IFA	BSL2
Russia	No	No[d]	ENIVD	Yes	No	PCR, ELISA, IFA	BSL3
Serbia	Yes	Yes[a]	ENIVD, EpiSouth	No	No	IFA, VI	BSL2
Slovakia	No	No	ENIVD	No	No	Referral	
Slovenia	Yes	No	ENIVD, CCH-FEVER, Arbo-Zoo-Net	Yes	No	PCR, ELISA, IFA, VI	BSL3+
Spain	Yes	Yes[e]	ENIVD, EpiSouth	Yes	No	PCR, IFA	BSL3
Sweden	Yes	No	ENIVD, CCH-FEVER, Euronet-P4, Arbo-Zoo-Net	Yes	Yes	PCR, IFA, VI	BSL4
Switzerland	Yes	Yes	ENIVD	Yes	No	PCR, ELISA	BSL4
Turkey	NA	Yes	ENIVD, EpiSouth, CCH-FEVER	Yes	No	PCR, ELISA, IFA	BSL3
United Kingdom	Yes	No	ENIVD, Euronet-P4	Yes (Porton Down)	Yes	PCR, ELISA, IFA, VI	BSL4

Arbo-Zoo-Net: Network for Capacity Building for the Control of Emerging Viral Vector Borne Zoonotic Diseases; BSL: biosafety level; CCH-FEVER: Crimean Congo Haemorrhagic Fever Network; ECDC: European Centre for Disease Prevention and Control; ELISA: enzyme-linked immunosorbent assay; ENIVD: European Network for Diagnostics of 'Imported' Viral Diseases; EpiSouth: Network for Communicable Disease Control in Southern Europe and Mediterranean Countries; Euronet-P4: European Network of Biosafety-Level-4 laboratories; EQA: external quality assessment; IFA: indirect immunofluorescence assay; NA: not available; PCR: polymerase chain reaction; QUANDHIP: Quality Assurance exercise and Networking on the Detection of Highly Infectious Pathogens project; VHF: viral haemorrhagic fever; VI: viral isolation.

[a] [22,23].

[b] National guides in preparation.

[c] [24].

[d] Not formal case definition [25].

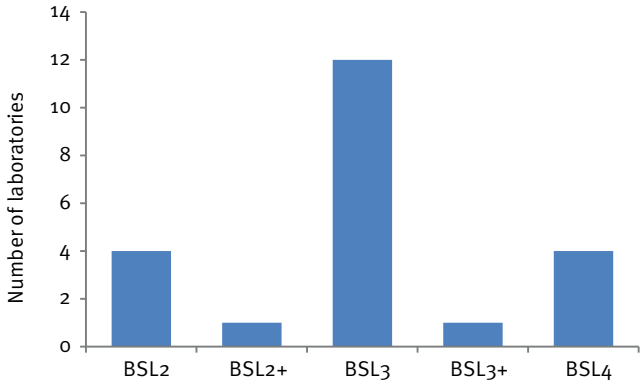
[e] [26]

[f] EU case definition for VHF [27].

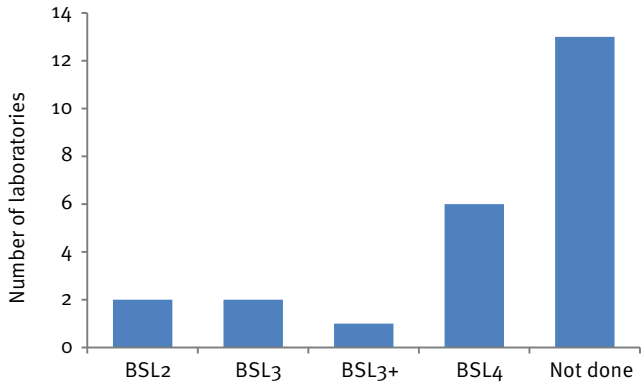
Figure 3

Biosafety levels for laboratories with Crimean-Congo haemorrhagic fever diagnostic capacities, ENIVD survey, 2012 (n=24)

A. For virus inactivation



B. For virus propagation



also participated in this EQA, in which 53 datasets were received from 44 laboratories worldwide, mostly European. Twenty of the datasets (38%) met the criteria with optimal performance.

The most definite way of CCHF diagnosis is detection of viral RNA combined with detection of IgM antibodies. Virus isolation as a diagnostic tool is rarely applied because high biocontainment laboratories (BSL4) are required. None of the European BSL4 laboratories are situated in CCHF areas, and among 11 laboratories performing viral propagation, five reported that they do not work in BSL4 facilities. Three of these five laboratories were in CCHF endemic countries.

In conclusion, the main priority issues to be addresses by European health authorities are: (i) establishing rapid and reliable protocols for CCHF laboratory diagnosis together with guidelines on storage, processing and transportation of samples, (ii) nominating a Regional Reference Expert Laboratory or a WHOCC in or near the endemic areas, and (iii) a comprehensive review of the BSL facilities suited to the reality in the endemic areas, their capacities and capabilities.

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Conflict of interest

None declared.

Authors' contributions

MDFG, AN, AT and LF conceived and designed the study; MDFG, OD, HZ, AN and LF designed the questionnaires; LF, MN and OD coordinated the collection of data through the ENIVD network; MDFG, AN and LF were involved in data management and analyses; AT, HZ, OD, MN and AP contributed with data analysis; MDFG and LF drafted the manuscript; all co-authors reviewed and assisted in the editing of the final version of the manuscript.

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