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Review

Biosafety standards for working with Crimean-Congo hemorrhagic fever virus

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In countries from which Crimean-Congo haemorrhagic fever (CCHF) is absent, the causative virus, CCHF virus (CCHFV), is classified as a hazard group 4 agent and handled in containment level (CL)-4. In contrast, most endemic countries out of necessity have had to perform diagnostic tests under biosafety level (BSL)-2 or -3 conditions. In particular, Turkey and several of the Balkan countries have safely processed more than 100 000 samples over many years in BSL-2 laboratories. It is therefore advocated that biosafety requirements for CCHF diagnostic procedures should be revised, to allow the tests required to be performed under enhanced BSL-2 conditions with appropriate biosafety laboratory equipment and personal protective equipment used according to standardized protocols in the countries affected. Downgrading of CCHFV research work from CL-4, BSL-4 to CL-3, BSL-3 should also be considered.

Introduction

Crimean-Congo hemorrhagic fever virus (CCHFV), a member of the genus *Nairovirus* of the family *Bunyaviridae*, causes a tick-borne zoonotic infection [Crimean-Congo haemorrhagic fever (CCHF)] in parts of Africa and Eurasia (Bente *et al.*, 2013). CCHFV has been classified as a hazard group 4 pathogen (UK) or risk group 4 (Europe, USA, international) in countries that have promulgated biosafety regulations, and should accordingly be handled in containment level 4 (CL-4, UK) or biosafety level 4 (BSL-4, Europe, USA, international) laboratories (Table 1).

Signs and symptoms after a sudden onset of disease, 1–7 days post-infection, progress from high grade fever, headache, fatigue, myalgia, abdominal pain, nausea, vomiting, diarrhoea, thrombocytopenia and rash, to haemorrhages from various body sites, shock and death in severe cases. Reported mortality rates vary widely from 2 to 30 % across studies and endemic countries (Ince *et al.*, 2014; Larichev, 2015).

Apart from transmission by tick bite as a major route of infection, transmission can also occur through handling or squashing of infected ticks, and contact with the blood of viraemic animals, or blood and other body fluids of patients. Consequently, livestock farmers, and abattoir and healthcare workers (HCWs) dominate the literature on reported infections. Nosocomial transmission to HCWs in close contact with patients in the acute phase have been documented throughout the endemic areas and are often linked to breaches of, or non-existent, barrier nursing techniques, or to percutaneous needlestick injuries (Tarantola *et al.*, 2007).

Following the occurrence of the first recognized outbreaks of 'Crimean haemorrhagic fever' in soldiers and displaced persons exposed to ticks while sleeping outdoors in 1944 and 1945, there were similar outbreaks associated with exposure of large numbers of people to ticks in major land reclamation or resettlement schemes in parts of the former Soviet Union, culminating in an epidemic in Khazakstan in 1989 (Hoogstraal, 1979; Lvov, 1994; Samudzi *et al.*, 2012). Subsequently, there were reports of a series of lesser outbreaks associated with exposure of people to blood and ticks from slaughter animals imported from Africa and Asia into the Near East (Williams *et al.*, 2000). Further large-scale outbreaks that occurred during the late 1990s and early 2000s involved exposure of war refugees to outdoor conditions in Kosovo, Albania, Macedonia and the Afghanistan–Pakistan border area (Duh *et al.*, 2007; Samudzi *et al.*, 2012). Finally, an outbreak of unprecedented magnitude emerged in Turkey in 2003, with 9787 clinical and laboratory confirmed CCHF cases by 2015. This outbreak has been ascribed to an increase in the tick population triggered by climate change, altered grazing practices and prohibition of the hunting of wild hosts of ticks (Estrada-Peña *et al.*, 2007).

Consequently, in recent years, the existing laboratory and health care facility infrastructure in south-eastern Europe and the Balkans, and especially in Turkey, has had to adapt to deal with a large influx of patients and samples potentially infected with a hazard group 4 pathogen.

The purpose of this paper is to review experiences of HCWs and scientists in handling CCHF patients and CCHFV-positive materials in order to derive safe recommendations for safe laboratory processing of known or suspected CCHFV-infected samples, and particularly at which biosafety level CCHFV material and samples from CCHF patients can be handled safely. First of all we re-appraise CCHF case fatality rates in endemic countries and in clinical cases. This is followed by a review of nosocomial infections and the most recent data from the large epidemic in Turkey, which indicate CCHFV is less easily transmitted from person to person than previously thought, as exemplified by seroprevelance

Table 1. CCHFV hazard groups and biosafety levels

Country	Endemic	Hazard group	Biosafety level of CCHFV diagnostics	Biosafety level of CCHFV research
South Africa	+	2	BSL-2 1980–2004 BSL-3 since 2004	BSL-4
Slovenia	–	4	BSL-2 1995–2004 BSL-3 since 2004	BSL-3 since 2004
Albania	+	2	BSL-2	BSL-3
Kosovo	+	2	BSL-2	BSL-2
Greece	+	4	BSL-2 1975–1987	BSL-3 glovebox introduced in 1987
Bulgaria	+	3	BSL-2	BSL-3
Serbia	+	n.i.	BSL-2	n.i.
Turkey	+	2	BSL-2	BSL-3 since 2012
Kazakhstan	+	4	BSL-3	BSL-4
Georgia	+	4	BSL-3	BSL-4
Iran	+	3	BSL-2 glovebox	–
Senegal	+	3	BSL-2 glovebox 1990–1999 BSL-3 2000–2015	BSL-3
Germany	–	4	BSL-4	BSL-4
Sweden	–	4	BSL-4	BSL-4
UK	–	4	BSL-4	BSL-4
France	–	4	BSL-4	BSL-4
USA	–	4	BSL-3	BSL-4

n.i., no information.

studies amongst HCWs dealing with CCHF patients, and is not transmitted into the community. We then turn to laboratory-acquired infections (LAI) while handling diagnostic or research samples and reveal that most infections were due to breaches of biosafety procedures in place and that a surprisingly high number of these infections had a mild or self-limiting course. Finally, we look at inactivation procedures for diagnostic samples to then formulate our recommendations for working with CCHFV.

Reported mortality rates and seroprevalences

Observed case fatality rates (CFRs) in CCHF vary from 2 to 30 % and are influenced by efficiency of diagnosis, cohort size sampled and speed of clinical intervention (Bente *et al.*, 2013; Ince *et al.*, 2014). Reported CFRs include 25 % from South Africa (Burt, 2015), 26 % from Kosovo (Humolli, 2015) and 15 % from Iran and Bulgaria (Sadegh, 2015; Christova, 2015). A structured epidemiological investigation in South Africa revealed that all or most infections in that country result in clinical disease (Fisher-Hoch *et al.*, 1992). Analysis of ProMED entries on CCHF from 1998 through 2013 reveals a CFR of 13 % among 3426 cases reported from Turkey, Russia, Iran, Pakistan and Afghanistan (Ince *et al.*, 2014). In South Russia, the CFR has decreased from 12–16 % (1953–1967) through 1.5–2 % (2006–2010) to 3.6–5.1 % (2011–2013). This is possibly due to an increased use of diagnostic kits and awareness of CCHF among medical staff (Larichev, 2015).

Following a regional epidemic in Turkey in 2003 and subsequent spread, 9787 cases with a CFR of 4.6 % were recorded by the end of 2015, which represents the highest number of cases on record (Korukluoglu, 2015). Studies in Turkey revealed a seroprevalence of 10–15 % in outbreak regions, with 88 % of infections appearing to be subclinical (Bodur *et al.*, 2012; Gunes *et al.*, 2009). The disease is often milder in children than in adults (Tezer *et al.*, 2010). Additionally, the circulation of CCHFV in endemic regions of Turkey is supported by serological studies on domestic and wild animals, with antibody prevalences reflecting the feeding preferences of the *Hyalomma* tick species that transmit the virus (Burt *et al.*, 1993; Fajs *et al.*, 2014; Mertens *et al.*, 2015; Mourya *et al.*, 2014; Shepherd *et al.*, 1987a, b).

CCHFV strain AP92 has been suggested to be less virulent than other CCHFV strains (Elevli *et al.*, 2010; Midilli *et al.*, 2009; Ozkaya *et al.*, 2010). It was initially isolated in 1975 (Papadopoulos & Koptopoulos, 1980) from *Rhipicephalus bursa* collected from goats in Greece, and AP92-like sequences have only recently been detected in ticks in Greece, Kosovo and Albania. A CCHFV AP92-like strain was also described in human cases in Turkey, but only causing mild CCHF (Midilli *et al.*, 2009; Ozkaya *et al.*, 2010). Recent data indicate a high CCHFV seroprevalence of up to 15 % in some CCHF non-endemic areas of Greece (Kastoria), possibly correlated to CCHFV-AP92 transmission by *R. bursa*.

This seems to be confirmed by recent data from Kosovo and Albania (Bino, 2015; Humolli, 2015; Papa *et al.*, 2014). The serological and epidemiological data support the initial assessment that CCHFV AP92 may be less pathogenic; however, there are no laboratory data to confirm this.

In contrast, after 13 years, the CFR in Turkey remains about 5 % despite major efforts to implement protection and prevention measures as well as public health training programmes and social mobilization (Bodur *et al.*, 2012; Korukluoglu, 2015; Ozkaya *et al.*, 2010; Uyar *et al.*, 2010).

Nosocomial CCHF infections

Nosocomial infections were recorded during the first reported outbreaks of 'Crimean haemorrhagic fever' in 1944 and 1945, and subsequently in other parts of the former Soviet Union and neighbouring countries (Hoogstraal, 1979). A more recent detailed literature review of nosocomial CCHF transmission to HCWs listed 44 infections in 494 HCW contacts in 12 countries (Tarantola *et al.*, 2007). Nosocomial infections were reported from South Africa (Joubert *et al.*, 1985; Shepherd *et al.*, 1985; Vandewal *et al.*, 1985; Vaneeden *et al.*, 1985a, b), Mauretania (Nabeth *et al.*, 2004), Sudan (Elata *et al.*, 2011), Albania (Harxhi *et al.*, 2005; Papa *et al.*, 2001), Kosovo (Papa *et al.*, 2002), Bulgaria (Kunchev & Kojouharova, 2008; Papa *et al.*, 2004), Turkey (Celikbas *et al.*, 2014; Gürbüz *et al.*, 2009), Iran (Chinikar *et al.*, 2013; Mardani *et al.*, 2009; Naderi *et al.*, 2013), Dubai (Suleiman *et al.*, 1980), Pakistan (Burney *et al.*, 1980; Hasan *et al.*, 2013), India (Yadav *et al.*, 2013), Tajikistan (ProMED-mail, 2009b), Kazakhstan (ProMED-mail, 2009a) and Germany (Conger *et al.*, 2015).

Nosocomial transmission often occurs during early illness before CCHF is recognized in the source patient, or where diagnostic laboratory capability is not available, and is usually associated with lack of, or improper use of, personal protective equipment (PPE). Once CCHF is recognized, nosocomial infection tends to occur most commonly where source patients manifest severe disease, probably because they develop the highest viraemias. Recent studies confirm that when a threshold of 10^8 viral genomes per ml of blood is exceeded, the disease progresses to fatal outcome (Cevik *et al.*, 2007; Duh *et al.*, 2007).

In general there is a very low CCHFV seropositivity in HCWs dealing with CCHF patients in Turkey (Akinci *et al.*, 2009; Ergonul *et al.*, 2007), and data on infections in HCWs in Turkey describe an up to 33 % risk of infection associated with needlestick injuries, and a 9 % risk after contact with body fluids (Tuna, 2015). In Iran, serological studies revealed a seroconversion rate of 3.8 % in HCWs exposed to CCHF patients. The seroconversion was 9.3 % in HCWs who had unprotected skin contact with body fluids and 7.1 % in those who suffered percutaneous injuries (Mardani *et al.*, 2007). A more recent study covering nine hospitals which managed 50 % of CCHF patients in Turkey from 2002 to 2014 found 51 HCW exposures by needlestick (62.7 %), splashes (23.5 %) and unidentified causes (13.7 %). Only 25 of these 51 exposures led to laboratory confirmed infections and four deaths (Leblebicioglu *et al.*, 2016).

High compliance to and proper use of PPE can indeed minimize the risk of infection as documented in a study from the Cumhuriyet University Education and Research Hospital in Turkey, were 1284 confirmed CCHF patients were treated between 2002 and 2012. The total seropositivity for CCHFV IgG was only 0.53 % in HCWs in infectious disease wards, which showed a high compliance to PPE of 100 %, 88.6 % and 82.9 % for gowns, gloves and masks, respectively (Gozel *et al.*, 2013). This is supported by another survey of 90 HCWs from three hospitals in the endemic regions which found a low seropositivity rate of 1 % (Bulut *et al.*, 2009).

Altogether, the clinical consensus is that simple barrier nursing and PPE can provide a good measure of protection to HCWs (Tarantola *et al.*, 2007). This is, for example, the case in the Ankara Ataturk Training and Research Hospital, were HCWs use contact protection (hand hygiene, gowns and gloves when needed). N95 masks and goggles are used only when dealing with patients with severe haemorrhagic symptoms in need of aerosol- and droplet-producing procedures such as aspiration and intubation. This pragmatic approach reduces full protection to the most severe cases from which nosocomial CCHFV transmission is most probable. Over the years, four doctors and three nurses had contact with infected blood and body fluids of CCHF patients, through needlestick injury, skin contact, contact to mucosal surfaces and probable aerosolization. All index cases were CCHFV PCR positive. The only HCW who developed seroconversion intubated an unconscious patient who had suffered a seizure. She was wearing gloves but no respiratory or eye protection.

In another incident, one HCW from the Ankara hospital forgot to don goggles when performing an emergency cardiopulmonary resuscitation (CPR) treatment of a severely ill CCHF patient. When injecting a drug, some blood squirted into her eye, which was immediately washed. Neither infection nor seroconversion resulted from the incident. Furthermore, no seroconversion was observed in any of the team performing the CPR without protective N95 masks (Z. Kocak Tufan, Turkey, personal communication).

Laboratory-acquired infections while handling patient samples

Modern diagnostic procedures usually comprise extraction of RNA from blood or other tissues of patients and the performance of a reverse-transcription (RT)-PCR, plus antibody tests on heat-inactivated serum. Culture of specimens for isolation of virus is performed less frequently.

Eight laboratory infections, one fatal, were recorded in Uganda during early investigations of 'Congo' virus in the

1960s, where known exposure of patients to infection occurred during the handling or processing of infected mice (East African Virus Research Institute Reports, cited in Swanepoel *et al.*, 1987).

A laboratory assistant infected himself while preparing plasma from a blood sample of a CCHF patient by centrifugation in 1986 in a laboratory in Rostov-na-Donu, Russia. The assistant developed a full-blown CCHF clinical picture including haemorrhages but survived after prolonged convalescence. A high initial CCHFV LD_{50} titre on day 1 and seroconversion were demonstrated (Gaidamovich *et al.*, 2000).

In South Africa, a clinical pathology laboratory technologist in a hospital in Kimberly was found to be seropositive for CCHF in 1986, but the presence of IgG antibody could not be conclusively linked to an earlier benign illness. The technologist routinely wore a laboratory coat and disposable gloves and performed all manipulations with blood and serum in class II cabinets. She used automated haematology and clinical pathology machines. A fatal case of CCHF occurred in 2006 in a technologist in a clinical pathology laboratory in Vereeniging, South Africa, who putatively only handled blood samples from a deceased CCHF patient in order to store them in a freezer. He had signed a procedure protocol which instructed him to wear a laboratory coat and gloves, but nobody observed him storing the samples. The technologist reportedly had not tested the samples, and it was never determined whether he had worn gloves or how he was exposed to infection, but virus isolates from the source patient and the technologist had identical nucleotide sequences. By the end of 2014, a total of 214 cases of CCHF had been confirmed in South Africa since the first case was recognized in 1981. The diagnostic tests involved the handling of 811 acute-phase blood samples at BSL-2 or -3 level with PPE (disposable gown, gloves, laboratory spectacles and N95 masks) without infections or seroconversions being recorded in the diagnostic laboratory, where the personnel regularly handle such specimens. The equipment used included class II cabinets, bench centrifuges, PCR thermocyclers, electrophoresis tanks, gel documentation readers, ELISA plate washers and readers and fluorescence microscopes. Mouse inoculation and tissue harvesting were performed in class II cabinets, and cages were held in a dedicated room with Hepa-filtered air supply and exhaust.

In contrast, the two laboratory infections reported above occurred in clinical pathology laboratories in hospitals where CCHF is infrequently encountered so that an adequate state of awareness is more difficult to maintain (all information on South Africa from R. Swanepoel, personal communication).

In Turkey, laboratory services issued instructions on the taking and shipment of samples, and made the information widely available on a web page (www.thsk.gov.tr). Shipments were strictly controlled, and, out of necessity, diagnostic assays were performed in BSL-2 laboratories. Samples had to be handled in class II biosafety cabinets using PPE (lab coat, gloves, goggles and NP95 mask) (Uyar *et al.*, 2010). Although a BSL-3 laboratory was finally opened in Ankara in 2012, it is not used for CCHFV diagnostics. At the time of drafting the present report, there had been 9787 clinical and laboratory confirmed cases of CCHF since 2003, and an estimated 90 000–100 000 samples had been processed under BSL-2 conditions (Leblebicioglu *et al.*, 2016). In some hospitals, CCHF blood samples are handled on the open bench by HCWs wearing gloves and N95 masks, but no goggles (Z. Kocak Tufan and C. Bulut, Turkey, personal communication). Two cases of LAI have been reported, one due to a needlestick while drawing blood and one due to handling a blood sample without wearing gloves (Leblebicioglu *et al.*, 2016).

Laboratory-acquired infections during research

In an incident in the Rostov-na-Donu laboratory in 1970, one of four staff members exposed to aerosols from a flask containing live virus that disintegrated in a centrifuge fell severely ill and died. In this instance, an underlying chronic hepatocholecystis may have contributed to the fatal outcome (Gaidamovich *et al.*, 2000).

In 1973, at the Institute for Epidemiology, Microbiology and Infectious Disease in Alma Ata (USSR, now Kasakhstan), a scientist preparing CCHFV antigen from suckling mouse brain using freon extraction fell severely ill and seroconverted but recovered. It was concluded that mixing volatile freon with the brain suspension may have caused formation of aerosols which were inhaled. As a consequence, work with volatile substances such as freon was required to be performed in chemical cabinets only (Karimov *et al.*, 1975).

In 1981, a virologist died in Cairo, Egypt, after mouth-pipetting a culture of a CCHFV isolate he had brought from Iraq (A. A. El-Sanousi, Egypt, personal communication).

At the Institute Pasteur de Dakar, two accidents were linked to handling suckling mice inoculated with a diagnostic sample and a tick pool suspension: in 1998, a technician suffered a needlestick accident, and in 1993, a staff member in breach of regulations handled cages with infected mice on an open bench without wearing any mask. They both fell ill, but experienced mild, self-limiting disease. Also in 1993, another technician was exposed to aerosols while preparing sucrose acetone antigen from infected suckling mouse brain since not all equipment was held in a laminar flow cabinet or in a BSL-3 laboratory. Again, the disease was self-limiting. A BSL-3 laboratory was built in Dakar in 1999. Henceforth, infected mouse cages were held in a special laboratory, and brain material was treated with beta propiolactone prior to use as antigen in routine ELISA for IgM/G antibody detection and immune ascitic fluid production in mice.

In 1999, a technician inflicted an abrasion on her hand with a needle during a CCHFV baby mouse brain passage

procedure in the National Center of Infectious and Parasitic Diseases laboratory in Sofia, Bulgaria. However, she was vaccinated with the Bulgarian CCHFV vaccine and presented with benign febrile illness only. In 2010, a Turkish laboratory worker in a university laboratory inadvertently poured a flask with a 10th passage CCHFV culture down the front of her lab coat but was not infected nor seroconverted (Aykut Ozkul, Turkey, personal communication).

Inactivation

Several publications have shown that chaotropic guanidine-isothiocyanate in commercial nucleic acid extraction buffers efficiently inactivates most enveloped viral agents including pox-, alpha-, bunya-, flavi- and filoviruses (Blow *et al.*, 2004; Smither *et al.*, 2015; Vinner & Fomsgaard, 2007).

Non-treated acute-phase serum samples of CCHF patients stored at 4 °C remain real-time-PCR positive for up to 30 days but the infectivity of these samples was not verified (A. Kubar, Turkey, personal communication). For serological analysis, diagnostic laboratories in Turkey and south-eastern Europe use thermal inactivation of serum at 56 °C for 30 min or even 45 min, although it was concluded in one study that 60 °C for 60 min is more effective for CCHFV (Mitchell & Mccormick, 1984).

In experiments performed recently in a South African laboratory to clearly analyse the conditions needed to inactivate CCHFV, CCHFV (strain SPU4/81) culture fluid with a titre of $1\times10^{7.6}$ $TCID_{50}\,ml^{-1}$ was incubated at 56 and 60 °C for 15, 30, 45 and 60 min and then inoculated into Vero E6 cell cultures. In all instances, virus growth was not detected. To show that the results were not due to the detection limit of the TCID assay at $1\times10^{1.5}$ $TCID_{50}\,ml^{-1}$, the inactivated suspensions were also inoculated intracerebraly into suckling mice (NIH strain) and all mice survived, even those inoculated with virus inactivated at 56 °C for only 15 min (Fig. 1). The experiments confirm that heat inactivation at 56 °C for 30 min used as a standard in Turkish (national guideline) and many other laboratories in south-eastern Europe is adequate for destruction of infectivity, and explains why LAI have not been reported from these diagnostic laboratories.

Biosafety regulations

The United Nations (UN) Convention on the Prohibition of the Development, Production and Stockpiling of Bacteriological (Biological) and Toxin weapons and on their Destruction (BTWC) as promulgated in 1972 imposed requirements on member states (party to the convention) for acquiring, holding, stockpiling, working with or disposing of certain pathogens (the list includes CCHFV) at specified biosafety levels, but BTWC lacked an organization or mechanisms to monitor and enforce compliance. Consequently, UN Security Council Resolution 1540 was passed in 2004 to enforce domestic compliance on states' parties as well as non-state actors through a 1540 committee. Purely diagnostic procedures and laboratories are exempted. It should be noted that documents such as the *Laboratory Safety Manual* (WHO, 2004), the *Biosafety in Microbiological and Biomedical Laboratories* manual (Service USDo-HaHSPH, 2009) and the European CEN Workshop Agreement 15793 (CWA 15793, 2011) only make recommendations on biosafety and do not impose legal requirements. Each country must promulgate its own biosafety legislation and regulations, and many have not yet done so. Consequently, there is wide divergence in the biosafety levels prescribed for handling CCHFV as some countries attempt to reconcile disease endemicity with laboratory capacity.

In a recent survey of laboratories in 28 countries that are members of the European Network for Diagnostics of Imported Viral Diseases (ENIVD), it was found that seven countries sent diagnostic samples for CCHF to reference centres elsewhere, while five tested samples in BSL-2 laboratories, 10 in BSL-3 laboratories and six in BSL-4 laboratories. Of 11 laboratories performing virus isolation and propagation, six did so in BSL-4 facilities and five in lower-grade facilities (Fernandez-Garcia *et al.*, 2012). Enquiries made for purposes of the present review revealed that in Slovenia, Turkey and Senegal, CCHFV diagnostic samples were handled at BSL-2 for years before a BSL-3 laboratory was finally available for research. In many other countries including Turkey, Kosovo, Albania and Bulgaria, diagnostics are still performed at BSL-2. Even in the USA,

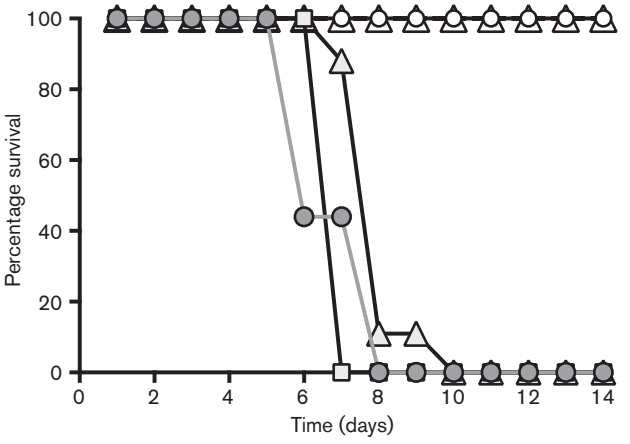

Fig. 1. Percentage survival of suckling mice injected intracerebrally with CCHFV-FBS mix ($1\times10^{7.3}$ $TCID_{50}\,ml^{-1}$). Dark grey circles: untreated CCHFV-FBS mix (positive control, n=9 mice). Grey squares: CCHFV-FBS mix treated at room temperature for 15 min (n=4). Grey triangles: CCHFV-FBS mix treated at room temperature for 60 min (n=9). White circles: CCHFV-FBS mix treated at 56 °C for 15/30/45/60 min (n=8/8/8/5). White triangles: CCHFV-FBS mix treated at 60 °C for 15/30/45/60 min (n=10/8/10/10). Please note that due to overlay only one line with white circles and one line with white triangles can be seen on the graph.

diagnostic samples are not handled in BSL-4 but in BSL-2 laboratories until the presence of CCHFV has been confirmed. In most non-endemic countries, diagnostic investigations, however, are conducted in BSL-4 facilities. All countries tend to use higher-grade facilities for research (Table 1).

Discussion

In non-endemic countries that coincidentally tend to be better resourced and can afford sophisticated laboratories, CCHFV is classed and handled as a hazard group 4 agent (Table 1). Agents in this group cause severe disease, are a serious hazard to staff, are likely to spread to the community and have no effective prophylaxis or treatment. In contrast, most endemic countries have perforce had to perform diagnostic tests under BSL-2 or -3 conditions, using appropriate PPE and laboratory practices. In particular, Turkey and several of the Balkan countries have processed large numbers of specimens without experiencing any LAI over many years. Although virological and seroepidemiological studies indicate that strains of virus circulating in the region may have reduced virulence, this alone does not account for the lack of LAI observed since monitoring for seroconversion confirms that transmission to HCWs is rare.

The present survey was performed to collect information on LAI in hospitals, and diagnostic and research laboratories. Only a few were identified. Such infections as have been reported in BSL-2 diagnostic laboratories almost invariably result from breaches of biosafety practise. Handling samples without gloves or mouth pipetting used in the initial isolations of CCHFV in the 1950s are no longer acceptable. Lessons have been learned from exposure to droplets in research settings, and in particular centrifuge buckets should be fitted with biosafety seals (clip on lids), and hazardous procedures should be performed in biosafety cabinets (Weidmann, 2012).

Outside of cabinets, culture flasks should be carried in sealed boxes, lids should be used on ELISA and culture plates during incubation, and film seals used for reading of plates. Sera should be heat-inactivated at 56 °C for 30 min before performing antibody tests.

Safety can be increased by wearing PPE commonly used in BSL-3 laboratories (face shield instead of goggles), without necessarily having to rely on positive pressure respirators. Accidental spillage of infected material unfortunately remains a possibility, but need not necessarily have a serious outcome as exemplified by the spill onto a Turkish laboratory worker. Animal inoculation procedures should preferably be avoided in diagnostic laboratories that do not have BSL-3 or -4 facilities. For BSL-3 laboratories, measures as implemented in Dakar are advisable.

There is, however, an ongoing debate on aerosol transmission of CCHFV in clinical settings. There are only a few reports describing the infection of close relatives of CCHFV patients, and these lack conclusive evidence of aerosol transmission (Saijo *et al.*, 2004; Tezer *et al.*, 2011). On the contrary, none of a cohort of 132 relatives staying with or visiting 88 CCHF patients of whom two patients died at the Cumhuriyet University Hospital in Turkey developed any symptoms nor seroconverted despite the fact that many did not comply with protective measures (Gozel *et al.*, 2014). The study indicates that CCHF is not easily spread between humans and into the community.

Although multiple-case incidents of nosocomial infection have been reported (Mauretania, Sudan, Pakistan; Tarantola *et al.*, 2007), there is no evidence for aerosol transmission in CCHF, and spread of infection was generally postulated to result from direct contact with body fluids or droplets of severely ill patients, percutaneous injuries and non-compliance with basic infection control precautions.

A recent review of possible aerosol (1–5 μm) or droplet (5–10 μm) transmission through coughing and vomiting in Ebola virus disease, concludes that there are no epidemiological data to support a large role for this mode of transmission, and that respiratory transmission (aerosol generation in the lung, exhalation and transmission by inhalation) does not occur (Osterholm *et al.*, 2015), and the same appears to apply to what is currently known about CCHF transmission. In contrast, aerosol transmission is well documented for smallpox virus and was conclusively shown by retrospective smoke experiments after isolated patients caused nosocomial transmission in Meschede in Germany (Wehrle *et al.*, 1970).

However, if actively generated, aerosols are indeed very likely to increase transmission, as recently described in a clinical setting due to the use of a compression inhaler to apply mucolytics and broncholytics to a CCHF patient while only surgical masks were used by HCWs (Pshenichnaya & Nenadskaya, 2015). In a most recent report, two HCWs suffered an infection, probably while using bag-valve-mask ventilation or performing bronchoscopies on an infected patient (Conger *et al.*, 2015). The obvious conclusion is to use fitted N95 masks if inhalation devices are used or aerosols might be actively generated in any other way. On the other hand, care has to be taken when using this type of mask, as unpublished data from Public Health England (N. Silman, UK, personal communication) show that the filter of N95 masks should not be used for more than 2 h as humidity trapped in the mask will bridge the filter, thus negating its efficiency.

In conclusion, diagnostic tests have been performed safely at BSL-2 level for many years in CCHF endemic countries that could not otherwise cope with demand. We therefore recommend that regulating authorities should revise biosafety requirements for CCHF diagnostic procedures to allow the tests required to be performed under enhanced BSL-2 conditions with appropriate biosafety laboratory equipment and PPE used according to standardized protocols in the affected countries (see Box 1). In this respect we would like to point out that class I cabinets which draw air away from the operator are preferable to class II cabinets

Box 1.

Recommendations for working with CCHFV

Primary containment

BSL-2 laboratory

Class BSL-2 laboratory class I/class II biosafety cabinet*

PPE

Lab coat

Gloves

Goggles/face shield

N95 mask

Additional procedures

Thermal inactivation of samples at 56 °C for 30 min

Guanidine-thiocyonate based nucleic acid extraction

Seal ELISA plates with transparent film before removing from biosafety cabinet

Use centrifugation buckets with clip-on lids, open buckets in biosafety cabinet only

*It is recommended to switch to class I cabinets if possible.

which provide a sterile working area through creating a laminar flow. Organizations such as the Centers for Disease Control and Prevention, USA, the National Institutes for Health, USA, the World Health Organization and the European Committee for Standardization should revise international recommendations accordingly. Technical advances arising from the successful deployment of mobile BSL-3 laboratories in the West African outbreak of Ebola disease (Chen *et al.*, 2015; Faye *et al.*, 2015; Inglis, 2015; Wölfel *et al.*, 2015) should be exploited to derive cost-effective improvements to diagnostic laboratories in the CCHF endemic countries. In particular, the use of flexible-walled or hard plastic glove boxes for extraction of nucleic acids and inactivation of sera would greatly improve laboratory safety. The evidence on LAI and LAI outcome, transmissibility and CFRs should merit discussion of the possibility of relaxing biosafety standards for research on CCHFV, and the graded application of isolation precautions in the treatment of patients according to clinical status should be codified.

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