Meeting report

Highlights and conclusions from the technical consultative meeting on novel coronavirus infection, Cairo, Egypt, 14–16 January 2013

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مقتطفات وخلاصات من الاجتماع التشاوري التقني حول العدوى بالفيروس التاجي الجديد؛ القاهرة، مصر، 14-16 كانون الثاني/ يناير 2013

كارل جوزيف، مامونار مالك، أنطوني واين ماونت، علي رضا معافي، سيلفي برياند، زياد ميمش، الفريق العامل للاجتماع على رواية الفيروس التاجي

الخلاصة: لقد أدى ظهور ذرية جديدة من الفيروس التاجي Coronavirus في شبه الجزيرة العربية إلى إثارة القلق الصحي على الصعيد العالمي عام 2012؛ وذلك يعود جزئياً إلى أن معظم حالات العدوى البشرية كانت مميتة، كما يعود جزئياً أيضاً إلى أنها من مصدر حيواني على ما يُفترَض. وقد عقدت منظمة الصحة العالمية في كانون الثاني/ يناير 2012، اجتماعاً عاجلاً في ظل المعلومات الضئيلة المتاحة حول السوابق الطبيعية والوبائية للعدوى بهذا الفيروس الجديد. وقد استعرض الاجتماع البيِّنات الراهنة، وحدَّد الفجوات الحاسمة في المعارف واللازمة لتحسين الوصول إلى فهمٍ أفضل لمخاطر الصحة العامة التي ترافق هذا الفيروس بحيث يتم تحسين التأهب كما تتم حماية ووقاية الصحة في العالم.

ABSTRACT The emergence of a novel strain of coronavirus in the Arabian Peninsula raised a global health concern in 2012, partly because the majority of human infections were fatal and partly due to its presumed animal origin. An urgent meeting of scientific and public health experts was convened by WHO in January 2013 in view of the limited knowledge available on the epidemiological and natural history of infection with this novel virus. The meeting reviewed current evidence and identified critical knowledge gaps to improve better understanding of the public health risk associated with the virus so as to improve preparedness and to safeguard and protect global health.

Faits marquants et conclusions de la réunion de consultation technique sur l'infection par le nouveau coronavirus, Le Caire (Égypte), 14 - 16 janvier 2013

RÉSUMÉ L'émergence d'une nouvelle souche de coronavirus dans la Péninsule arabique a soulevé des inquiétudes sanitaires à l'échelle mondiale en 2012, d'une part parce que la majorité des infections humaines ont été mortelles et d'autre part parce qu'une origine animale était suspectée. L'Organisation mondiale de la Santé a invité des experts en santé publique et scientifiques à une réunion urgente en janvier 2013, étant donné les connaissances limitées disponibles sur l'évolution épidémiologique et naturelle de l'infection par ce nouveau virus. Pendant la réunion, les données disponibles ont été examinées et des lacunes critiques dans les connaissances ont été identifiées en vue d'améliorer la compréhension du risque pour la santé publique associé à ce virus, d'intensifier la préparation et de préserver et de protéger la santé mondiale.

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Introduction

In 2012, a multi-country outbreak of severe acute respiratory disease in the Arabian Peninsula, caused by a novel coronavirus (nCoV), raised a global health alert [1–3]. In light of the severity of illness seen amongst the laboratory-confirmed cases reported to the World Health Organization (WHO) by January 2013, where the mortality rate was over 55% [4], the discovery of this new virus triggered unprecedented global attention.

This novel virus brought back memories of the global epidemic of severe acute respiratory syndrome (SARS) in 2003 as this new virus was presumed to be of animal origin and also belonged to the same family of Coronoviridae as that of the SARS virus (SARS–CoV). Clinical symptoms caused by nCoV infection also matched the clinical picture of acute primary viral pneumonia seen in many patients suffering from SARS [5].

The genetic sequence data indicated that this new coronavirus was a beta-coronavirus similar to bat coronaviruses, but not similar to any other coronavirus previously described in humans, including the coronavirus that caused SARS in 2003 [6–8]. The available evidence related to this nCoV continued to suggest a zoonotic origin for the virus, and experience with the SARS–CoV also showed that any novel virus that is zoonotic in origin has the potential to efficiently transmit from person to person, especially in healthcare settings, and to cause a global pandemic of severe human illness.

An urgent meeting of scientific and public health experts involved in the national and global investigations of this nCoV infection was convened by WHO at its Eastern Mediterranean Regional Office in Cairo, Egypt, from 14 to 16 January 2013. The meeting brought together WHO, national experts who had been involved in investigations around the cases that have occurred, scientists who had been involved with the study of the origin of the virus, and experts who had participated in the global public health response to SARS and the avian influenza epidemic. The purpose of this meeting was to review and discuss the scientific and public health understanding of the emergence of nCoV to date, identify critical knowledge gaps in understanding the current risk and identify the next steps to improve knowledge and close up the research gap for public health action at the national and international level. The meeting was organized in 5 thematic sessions and in each of these sessions, available scientific information and up-to-date evidence on nCoV were presented and discussed by the participants. The sessions included (i) epidemiological information; (ii) virological and animal investigation; (iii) development of tests for nCoV; (iv) experience from SARS; and (v) risk communication and preparedness. The final session of the meeting on "knowledge gaps and priorities for research" reviewed the currently available scientific evidence and identified the critical knowledge gaps that needed to be addressed in order to improve global understanding of the risk associated with this novel virus. This paper summarizes the currently available information on the virus, knowledge gaps and the priority public health research activities that were discussed in the meeting in order to improve global preparedness for any potential pandemic caused by this novel virus.

Highlights

Environmental and epidemiological investigation

By December 2012, 6 laboratory-confirmed cases of nCoV, including 3 deaths, had been reported from Saudi Arabia. Three (3) cases occurred sporadically and 3 were part of a family cluster. The dates of onset for the 3 sporadic cases were 6 June—10 October. The dates of onset for the cases within the family cluster were sequential and ranged from 5 October to 3 November, consistent with human-to-human transmission. No community transmission was reported.

The investigation of potential environmental and animal sources of the nCoV carried out in the Bisha area of Saudi Arabia in September 2012 was not conclusive. Bats and dates were the main focus of investigation. Bats were targeted because of the genetic relatedness of the nCoV to coronaviruses previously found in bats in Mexico and elsewhere [9]. Bat CoVHKU9, bovine respiratory CoV and Kenya Idoline bat viruses were detected among the 755 oral, rectal and serological samples from insectivorous bats collected in the Bisha area of Saudi Arabia and tested in the United States of America (USA) in October. Sequencing by nested polymerase chain reaction (PCR) showed that Saudi bat virus was genetically indistinguishable from the nCoV identified by the Erasmus Medical Center (EMC) of Rotterdam in the Netherlands. No virus was cultured from the Bisha bats. No microbiological or environmental link was made between date farming and date consumption for the first confirmed case of nCoV infection and no tests were carried out on camels or goats. Though the inability to culture the virus or extend the sequence makes interpretation of the results difficult, it did suggest that the virus may have been in the environment at very low frequency and concentration. It remained unknown whether the virus was in livestock or another intermediate host, nor how it may have been transmitted to humans.

The first case reported from Qatar had onset of illness on 3 September with a travel history to Mecca from 29 July to 18 August. He had no known contact with sick people. He owns a farm with camels and sheep which he visited once on his return from Mecca. The patient was transferred to the United Kingdom

(UK) on 12 September where the nCoV was identified through culture and PCR. Genetic sequencing indicated that the virus was the same as that previously found in the patient in Saudi Arabia. An investigation of health-care workers exposed to the patient identified 4 who became symptomatic after exposure, but laboratory reports were negative for all these cases when tested at the Health Protection Agency (HPA) in London.

The UK Health Protection Agency (now known as Public Health England) confirmed on 22 September 2012 infection with a novel coronavirus in a patient in a London hospital who had been transferred from Qatar 11 days previously. Following the institution of strict respiratory isolation, infection control procedures and enhanced surveillance as a response to this imported case from Qatar, 64 contacts (56 health-care workers and 8 family and friends) were identified. Ten days after last exposure, none of the 64 had developed severe disease; 13 of them had reported mild respiratory symptoms during the 10-day follow-up period. The novel coronavirus was not detected in 10 of 10 symptomatic contacts tested. The health-care workers had a variety of exposures, including some during an aerosol generating procedure. Preliminary results of serological testing found no evidence of a serological response consistent with infection among close contacts of the confirmed case.

The second case from Qatar was a single male and owns a camel and goat farm. He had no contact with the animals, had no history of travel, and no known contact with sick people. He was treated in 2 hospitals in Qatar from 12 to 24 October. Respiratory samples— nasal swabs and an endotracheal tube (ETT) aspirate were collected from the patient on 13 and 17 October and were negative when tested for a standard panel of respiratory viruses in Qatar. The ETT sample sent to the HPA in

London tested positive for nCoV. The patient was transferred to Germany on 24 October. The estimated number of health-care workers and family contacts associated with the case was 85. Respiratory and serum samples were collected from 20 of these contacts and the nCoV was not detected in any of the respiratory samples.

An investigation of 123 contacts (120 hospital and 3 out-of-hospital contacts) of the first nCoV case identified in Germany was conducted in November 2012 at the Robert Koch Institute in Berlin, Germany, to evaluate human-to-human transmission. The case was a patient with acute respiratory distress syndrome of unknown origin and symptom onset on 5 October who was transferred from Qatar to a specialist lung clinic in Germany. Eighty-five contacts provided blood for a serological test and analysis was performed using a 2-stage approach with an initial immunofluorescence assay as a screening test, followed by recombinant immunofluorescence assays and an nCoV-specific serum neutralisation test. None of the contacts tested positive for antibodies to the nCoV, indicating that no transmission had occurred to contacts after 20 days post exposure.

A cluster of severe acute respiratory infections occurred in April 2012 in a health-care setting in Zarqa, Jordan. In November 2012, retrospective laboratory investigation of a stored specimen of bronchoalveolar lavage and a serum sample confirmed nCoV infection in 2 patients, both of whom died. Additionally, 11 probable cases were identified through a retrospective epidemiological investigation. Ten of the 13 persons in this cluster were health-care workers. No sustained community transmission was observed.

Virological and animal investigations

Pathogenesis of nCoV

A study conducted at the University of Bonn found that the cellular

receptor for nCoV is different from that used by the SARS–CoV, which also causes Severe Acute Respiratory Syndrome [10]. It has been previously shown that changes in the spike protein (S1) of the coronavirus are an important means by which the virus adapts to a new host. The S1 protein as the presumed binding site in nCoV was cloned and tested for binding to target cells and was found to bind to human cells, and in animal models. The dipeptidyl peptidase 4 (DPP4) protein, an enzyme expressed on the surface of some cells, has now been identified as a receptor to which the S1 virus protein binds in human and bat cells.

Further studies are now going on in the animal model related to pathogenic potential of nCoV in human respiratory tissues

Sequencing studies

The complete genome sequence of the nCoV-EMC/2012 virus has been published [8]. By January 2013, only 2 viruses were fully sequenced: the nCoV-EMC from Saudi Arabia and the one from Qatar sequenced by the HPA in London (England1_CoV). The analysis of 3 sequenced genomic fragments showed that the genetic structure of the virus isolate in Germany was identical to the virus isolated from the patient in Qatar in the UK.

Methods for detecting nCoV by 2 real-time, reverse-transcription PCR assays (real-time RT-PCR) were published in September 2012 [6]. Two target regions were chosen for screening, confirmation and sequencing of the nCoV; the Upstream of the E gene (UpE) for screening and ORF1b for confirmation.

A 700-nucleotide segment of the N gene of the virus from one of the cases in Jordan sequenced at the United States Naval Medical Research Unit-3 (NAMRU-3) had 99% homology to the EMC and London viruses.

Development of laboratory tests for nCoV

Tests by real-time PCR

The current WHO interim guidance on PCR testing for nCoV states that a positive PCR test should be followed by a confirmatory test with the 1A assay or by sequencing target sites within the nucleocapsid protein region of the genome [11]. Positive control materials developed by the University of Bonn are available for the upE and 1A tests. Two new assays for sequencing, particularly where an insertion/deletion polymorphism might exist, as is the case between the EMC virus and the London virus, were also made available in December 2012. The assays are now available commercially worldwide and have been provided to some of the countries of the WHO Eastern Mediterranean Region where cases have occurred. The Centers for Disease Control and Prevention (CDC), USA, has developed two nucleocapsid assays for detection of nCoV. These assays were distributed to Saudi Arabia and Jordan, and were used by NAMRU-3 to confirm the cases in Jordan.

Serological tests

Two immunofluorescent antibody (IFA) detection assays have been developed; one using conventional IFA methods and the other a rapid method. Confirmatory tests for positive IFA results are carried out through recombinant subunit assays, plaque reduction neutralization tests, and western blot to rule out false positive results [6]. Tests for sensitivity and cross-reactivity against other coronaviruses and other respiratory viruses with the nCoV-EMC found no cross-reactivity using these confirmatory methods. Further validation studies are needed and the IFA test is not suitable for broad population screening of asymptomatic individuals. Many serological samples are in storage from the 9 nCoV cases and their contacts and these could be used to further validate the IFA tests.

The UK HPA tested 124 sera samples from family and health-care worker contacts of the imported case from Qatar. The sera were screened for reactivity to coronavirus NL63 (CoV gp1), OC43 (CoV gp2a), SARS (CoV gp2b) and nCoV England/1 2012 (CoV gp2c). No reactivity with the nCoV England 1 antigen was detected. When recombinant assays were tested for cross-reactivity with these strains and bat CoV strains from groups 1, 2a, 2b, 2c and 2d, the group 2c bat strains were reactive to the London patient sera. These results were part of work in progress, and further studies will be needed to interpret the data. Cross-reactivity, particularly in older people who have been exposed to different coronaviruses over time, can be natural. Cross reactions might also occur when detecting low titres that might be a result of past infection combined with a fresh infection of a different CoV. A wide range of tests are required to fully understand these issues.

Ecological studies

Ecological studies have shown that bats are natural hosts of 2b and 2c beta-coronavirus subgroups. Extensive bat sampling has been carried out in Africa and Europe, where a high percentage of bats among the *Nycteris* (25%) and *Pipistrellus* (36%) genera were found to be positive with CoV of the 2c clade. The pipistrellus bats inhabit the Arabian Peninsula but also migrate to Africa and other continents. The knowledge that insectivorous bats are natural hosts for betacoronaviruses, and that coronaviruses have been detected in a significant proportion of bats, does not in itself provide definitive evidence that they are the source of infection for the cases of nCoV detected in the Arabian Peninsula. More studies are needed to ascertain whether a virus even more closely related to nCoV-EMC might be found in other species and whether other animals might be sources or carriers of the novel human coronavirus.

Experience from SARS

The global experience from SARS was a stark reminder of how a novel virus can cause a global public health emergency, from a few sporadic cases to a worldwide epidemic. The SARS-CoV spread to 5 countries within a 24-hour period, before international public health measures were in place to identify, control and prevent the spread of infection. The importance and challenges in the implementation of public health measures such as strict hospital infection control measures, case identification, and comprehensive identification and quarantine of contacts cannot be over-emphasized. Sharing of information at the local, national and international level was key to managing public and professional fear and anxiety. The lessons learned from the SARS outbreak in 2003 emphasized the need to strengthen international health regulations, have national plans in place to handle similar future outbreaks, maintain public health epidemiological and microbiological capacity and keep ahead of the curve.

Risk communication and preparedness

The national and international public health organizations have kept the public fully apprised of the emergence and investigation of the nCoV cases on their websites through regular bulletins and updates, question and answer sheets, or through publications such as the rapid reports in *Eurosurveillance*. The WHO has 3 main communication channels: the Event Information Site (EIS), a password-protected rapid reporting system; the disease outbreak news (DON), which is a public system; and traditional media through its website and publications. The important lessons learned were mainly related to timely and adequate dissemination of information—the more information the better—to help the affected country, to help other neighbouring countries, and to help the world be better prepared.

Gap analysis and priorities for future research and investigation of novel coronavirus

A number of key knowledge gaps were identified to better understand the evolution and public health risk associated with this nCoV. The most important ones were the source of the virus, the exposures that resulted in human infection, and the mode of transmission. Those areas where more information is needed to characterize the current and future risk posed to global health by this novel virus are:

- source of the infection and its animal reservoir(s),

- extent of geographical spread,

- route(s) of transmission,

- transmissibility of infection and degree of infectiousness,

- exposure patterns,

- incubation periods,

- pathogenesis including age and gender determinants,

- clinical spectrum of severe illness and evidence of mild infections,

- what further diagnostic tests should be developed for detection of virus,

- interpretation of test results.

Four key areas were highlighted for guiding future efforts in furthering the global understanding and knowledge of nCoV. These included (i) defining the geographic extent of virus transmission, (ii) detecting any escalation in incidence, (iii) improving the case definition; and (iv) identifying the source of infection. A number of recommended actions were also proposed and future steps were identified for WHO's role. Table 1 presents a summary of these priority research areas and the suggested recommended actions to improve the current knowledge gaps.

Conclusion and next steps

The emergence of new, infectious, global threats in the past 4 decades (e.g. AIDS, avian influenza A/H5N1 and SARS) has reshaped thinking at both national and international levels on the nature and level of public health responses needed for these threats. *The International Health Regulations (2005)* have emphasized that all countries are at risk from new infections and therefore need to collaborate on information sharing and data exchange when they occur [12]. In the current age of immediate and ongoing access to world-wide digital information, there are high expectations globally that everything is being done to detect and control an emerging disease threat. Uncertainties as to how a newly discovered disease is going to evolve means that preparations have to be determined at both the national and the global level.

During the Cairo meeting, all current knowledge about the cases of nCoV infection and about what has shaped priorities for future actions at the national and the international level was evaluated. After consolidating information on current scientific activities ongoing at the global level, the delegates identified activities that would advance knowledge about this infection in the immediate and long term. These include:

- preparing an inventory of current nCoV virological research activities in European, American and other national laboratories;

- encouraging cooperation in the development of virological studies;

- preparing an inventory of training opportunities for laboratory staff testing for nCoV;

- preparing an inventory of laboratories developing nCoV serological tests and list which laboratories are using which tests;

- preparing an inventory of countries willing to test cases of severe acute respiratory infection (SARI) for nCoV;

- liaising with the animal research group to strengthen collaborative studies and coordinate future studies into the reservoir of infection for nCoV;

- itemizing all collaborating resources and identifying where capacity building is required;

- ensuring that risk assessment guidelines, infection control guidelines and other documents are regularly reviewed and communicated to those that need to know.

Technical working group members

The members of the technical working group include: Christian Drosten, Walter Ian Lipkin, Ron Fouchier, Udo Buchholz, Keiji Fukuda, Jaouad Mahjour, Hala Esmat, Dalia Samhouri, Gregory Hartl, Agnus Nicoll, Richard Pebody, Maria Zambon, Theresa Tam, Barbara Raymond, Hamad Eid Al-Romaihi, Said Hamed Al Dhahry and Sultan Mabdalla.

Table 1 Priority research area and recommended actions to improve global understanding and knowledge on novel coronavirus infection

Priority research area	Recommended actions	Next steps, with support from WHO
Defining the spectrum of disease severity (extent of virus transmission)	• Develop a standardized and sensitive approach for seroepidemiological studies to identify severely and mildly ill persons, including those who may have been exposed to the virus but remained well. The studies should focus on: • patients with SARI; • close contacts of cases who become symptomatic; • clusters of SARI occurring in occupational groups, particularly health care workers; • Standardize and validate laboratory methods for serological assays (IFA or ELISA) to be used for screening for more specific and conclusive results.	• Create a study group and framework for designing appropriate studies using standardized epidemiological methods. • Identify and prioritize groups for testing. • Develop protocols for standardized serological methods. • Encourage sharing of clinical samples between countries and laboratories. • Initiate serosurveys of contacts and probable cases to elucidate the full clinical spectrum of the disease, mindful of cross reaction. issues in serological studies. • Initiate laboratory training programmes for using standard methods.
Detecting any increase or decrease in incidence of infection	• Establish hospital baselines for pneumonia and monitor any unexplained rise in trend. • Enhance surveillance within groups such as case contacts, health care workers and clusters of patients with severe respiratory illness. • Prospectively collect and test sputum specimens from SARI patients and their close contacts.	• Develop standard surveillance tools for monitoring changes in rates of pneumonic illness or detection of illness in selected population groups. • Develop a standard protocol for guidance on types of specimens to collect from selected population groups. • Ensure countries test single cases of unexplained severe respiratory illness and report positives to WHO.
Improving the case definition	• Collect more data on the clinical spectrum and natural history of nCoV to inform changes to the case definition (implementing a 2-stage case definition of initial screening followed by closer examination of cases that meet specific criteria can be considered). • Utilize protocols from the SARS epidemic to develop risk factor studies.	• Contact affected countries to request data to better define key clinical features of known cases. • Obtain clinical information on known cases: pool all information from affected countries using a standardized extraction form. • Develop a global case definition for reporting. • Continue to monitor the effectiveness of the case definition and revise when relevant.
Identifying the source of infection	• Conduct animal studies to inform sources; (protocols from the studies carried out for the SARS epidemic could be utilized for developing risk factor studies into nCoV infection).	• Include animal studies in the framework.

ELISA = enzyme-linked immunosorbent assay; IFA = immunofluorescence assay; nCoV = novel coronavirus; SARI = severe acute respiratory infection; SARS = severe acute respiratory syndrome; WHO = World Health Organization.

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