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Investigating outbreaks of initially unknown aetiology in complex settings: findings and recommendations from 10 case studies

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Background: Outbreaks of unknown aetiology in complex settings pose challenges and there is little information about investigation methods. We reviewed investigations into such outbreaks to identify methods favouring or impeding identification of the cause.

Methods: We used two approaches: reviewing scientific literature and soliciting key informants. Case studies were developed through interviews with people involved and triangulated with documents available from the time of the investigation.

Results: Ten outbreaks in African or Asian countries within the period 2007–2017 were selected. The cause was identified in seven, of which two had an unclear mode of transmission, and in three, neither origin nor transmission mode was identified. Four events were caused by infectious agents and three by chemical poisoning. Despite differences in the outbreaks, similar obstacles were noted: incomplete or delayed description of patients, comorbidities confounding clinical pictures and case definitions wrongly attributed. Repeated rounds of data collection and laboratory investigations were common and there was limited capacity to ship samples.

Discussion: It was not possible to define activities that led to prompt identification of the cause in the case studies selected. Based on the observations, we conclude that basing case definitions on precise medical observations, implementing initial comprehensive data collection, including environmental, social and behavioural information; and involving local informants could save precious time and hasten implementation of control measures.

Keywords: communicable diseases, data collection, falsified drugs, low- and middle-income countries, outbreak, poisoning.

Introduction

Epidemiologists and public health professionals charged with investigating outbreaks of unknown aetiology face many common challenges, whether the outbreaks occur in well-resourced or low-resourced areas.[1] Little is known about outbreaks that occur in remote settings other than what is highlighted in the global scientific literature. A review of ProMed reports of undiagnosed disease events shows that those events mainly occurred within low-resource countries.[2] New, emerging infectious diseases also present first as outbreaks of unknown aetiology, as in the first cases of acquired immunodeficiency syndrome, severe acute respiratory syndrome (SARS), Middle East respiratory syndrome and coronavirus disease 2019.[3] From our experience and literature review, little information is available to the scientific community about outbreaks where the cause is not identified or where there is controversy around the primary agent involved. Also, detailed information regarding the investigation processes in such

outbreaks is scant: what challenges do investigation teams face for data collection, sample collection, storage and shipment? Even less well documented is how to propose control measures while there is still uncertainty about the cause of the outbreak.

In 2012 Goodman et al.[1] published a review of 14 historically important syndromic outbreaks initially of unknown aetiology investigated in the USA and Cuba. The authors identified lessons learned from which they derived a series of necessary measures, many of which are not always available in remote settings.

Dalton et al.[4] proposed a framework for auditing outbreak investigations in Australia and insisted on the establishment of performance standards in outbreak investigations. However, the resources allocated to investigations vary dramatically from one country or setting to another and make the identification of such criteria difficult. Even so, the need for good practices in investigating these outbreaks supports the work presented here.

As part of the World Health Organization (WHO) Outbreak Toolkit project, we initiated a review of recent investigations into outbreaks of unknown aetiology. The Outbreak Toolkit project is a WHO-coordinated initiative aimed at improving the outcomes of investigations into outbreaks, of known or unknown aetiology, by developing one website with tools and guidance to assist the public health professionals who respond to them (https://www.who.int/emergencies/outbreak-toolkit). Special attention was paid to outbreaks for which investigations did not lead to the determination of the aetiology and those that occurred in remote or complex settings (especially conflict and hard-to-reach areas), in humanitarian crises, or in low- and middle-income countries (LMICs). The aim of this work was to describe the process of investigation of the outbreaks of unknown aetiology occurring in complex settings and to determine whether certain methods, techniques or strategies of investigation aided or impeded the rapid identification of the aetiology of the outbreak. In this article we present a summary of 10 case studies and discuss key findings that could assist the development of a framework and recommendations for investigating outbreaks of unknown aetiology.

Methods

Definition of an outbreak of unknown aetiology

For this study, an outbreak of unknown aetiology refers to either an outbreak where a definitive causative agent was not identified during an initial investigation or an outbreak where a definitive causative agent was identified during an initial investigation but the source or route of transmission remained unclear. For the purpose of the study, we classified the outcome of the investigations as:

- Complete outcome: the causative agent, its source and mode of transmission were identified.
- Incomplete outcome: the causative agent was identified, but the source or mode of transmission or what triggered the outbreak remained unclear.
- Unsatisfactory outcome: an underlying causative agent has been proposed but remains disputed or unconfirmed and no clear source or mode of transmission could be established.

Selection of the case studies

We began by compiling a list of investigations into public health 'events' of unknown aetiology that occurred within a 10-y period from 2007 to 2017. We used two approaches: searching the scientific literature, specialized social media and a WHO repository of alerts, using standardized keywords described below; and key informant interviews with representatives of institutions involved in investigations into outbreaks of unknown aetiology.

We conducted the search of scientific literature in the PubMed database using the keywords: ('Disease Outbreaks'[Mesh] OR 'disease outbreak'[TW] OR 'disease outbreaks'[TW]) AND (unidentified [TW] OR 'unknown causes'[TW] OR 'unknown cause'[TW] OR mysterious [TW]) using the English language. The search of the ProMed (https://www.promedmail.org, accessed 22 December 2019) and FluTrackers.com archives (https://flutrackers.com/forum/, accessed 22 December 2019) was conducted using the keywords 'undiagnosed', 'unknown' 'unknown origin' and 'mysterious'. The records of the WHO Event Management System were also searched for files relating to outbreaks of unknown origin (grey literature). Fig. 1 illustrates the search strategy and selection process for the 10 case studies.

An important criterion for case study selection was the availability of health professionals involved in the investigation to share first-hand information about the process of the investigation into the outbreak.

Source of information

Ten case studies were developed through the collection and review of primary and secondary documents from the investigations, including outbreak reports and outbreak situation reports, line lists, media articles, peer-reviewed journal articles and laboratory reports. The complete list of documents consulted is available in the Annex (Other sources of information). Key informant interviews with health professionals directly involved in the investigations were conducted using a semistructured interview guide.

Analysis

For each of the case studies we tracked the factors and activities that either promoted or impaired the progress of the investigation towards the outcome. Investigations that identified clear causative agents were compared with those that did not.

Definition

The term 'transnational institution' describes any organization that has a presence in more than one country and routinely draws on material resources and expertise of more than one country.

Results

Description of the ten outbreaks

The 10 case studies reviewed are summarized in Table 1.

Outcome of the investigations

A clear causative agent was identified in 7 of 10 case studies. In five of these, both the source and the route of transmission were identified (complete outcome) (Table 2). In the remaining two

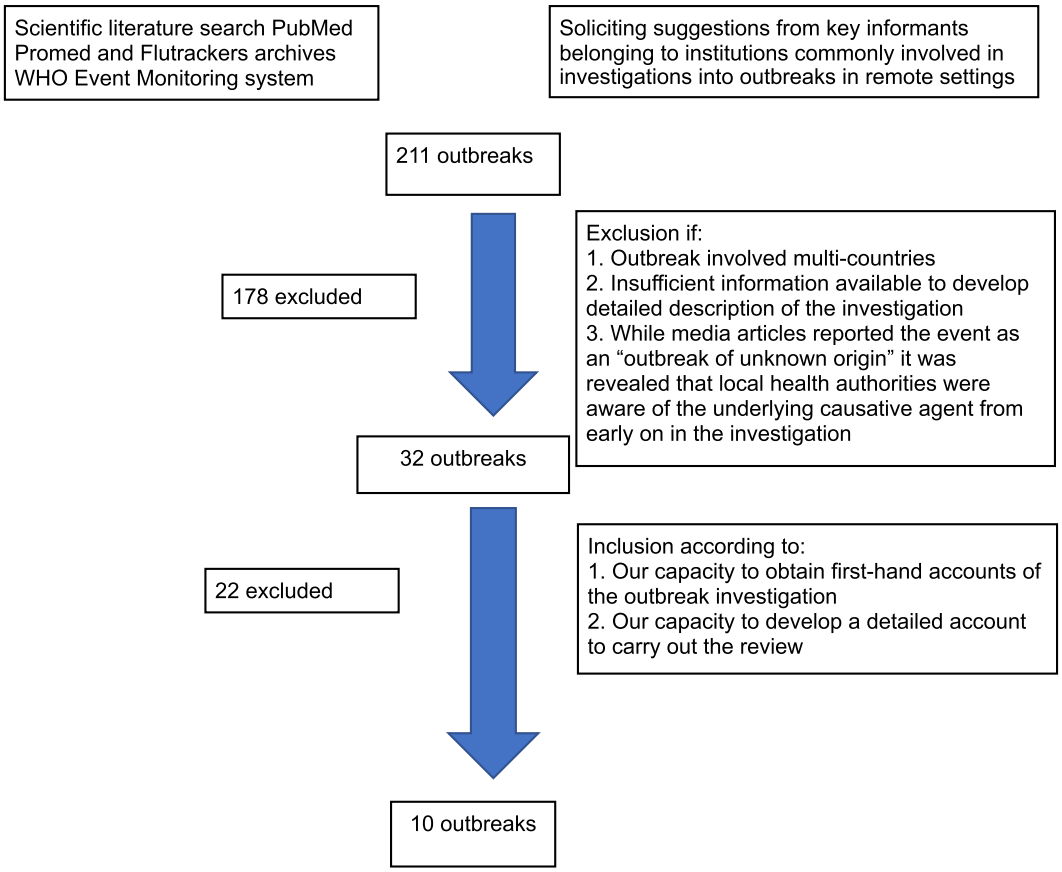

Figure 1. Selection process of the 10 cases studies for the review of investigations of outbreaks of initially unknown aetiology in complex settings.

case studies, a compelling causative agent was identified but the event remained unexplained (incomplete outcome). These case studies were classified as incomplete because in Liberia there was no explanation for the sudden clustering of unusual forms of meningococcal disease and septicaemia, and in Cambodia there was no definitive explanation for the unusually high case fatality ratio (CFR) observed in the enterovirus 71 outbreak at the centre of the case study. For the three remaining case studies, Vietnam, Congo and São Tomé and Príncipe, while the outbreaks have at times been attributed to various infectious and non-infectious agents, there is no consensus by the partners involved in the investigation about the agent primarily driving the event (unsatisfactory outcome).

Public health intervention

Five investigations [Angola, Nigeria, Democratic Republic of the Congo (DRC), Liberia and Cambodia] resulted in public health interventions or general awareness raising that contributed to the early conclusion of the acute events. In Cameroon, the investigation lasted for 2 y before a plausible aetiology was identified and a control programme implemented. In São Tomé and Príncipe and Ethiopia, the combination of messages and awareness of the population about hygiene, in conjunction with favourable climatic factors, likely led to the resolution of the event. We were unable to document with precision whether the events in Vietnam and Congo had been resolved by 2022.

Place and magnitude

Eight of the ten case studies occurred in African countries and two in East Asia. All the events occurred in LMICs, with seven occurring in impoverished, remote and difficult-to-access communities. The magnitude of the outbreaks varied significantly: the largest outbreak extended over an entire country, two involved poor suburbs of a capital city, six affected villages in remote areas and the smallest outbreak was restricted to a suburb of a district capital. The number of cases reported varied from 30 to >3000.

Syndromic categories

We observed three case studies with neurological syndromes, one with gastrointestinal syndrome associated with neurological manifestations, one with severe respiratory syndrome with neurological manifestations, three with cutaneous syndromes, one with fatal febrile syndrome and one with acute jaundice syndrome. The severity of the event, based on CFR, varied from an estimated 0 to 98% (Table 1).

Table 1. Main characteristics of the selected case studies

Country	Year first reported	Geographical distribution	Approximate number of cases	Population affected	Reported CFR	Reported comorbidities	Agent	Source and mode of transmission	Outcome
Angola	2007	Municipality	500	Women and children	0%		Sodium bromide	Table salt adulterated with sodium bromide: subacute prolonged intoxication	Successful intervention
Nigeria	2010	Several districts	300 children <5 y old	Mainly young children, but entire population affected	48% among children	Malaria infection increased neurotoxicity of lead	Lead	Environmental pollution caused by informal gold mining activities	Successful intervention, but mining practices causing contamination remains a challenge
DRC	2014	Several districts	1000	All ages	0%		Haloperidol	Counterfeit diazepam tablets containing haloperidol	Withdrawal of agent, WHO alert released
Ethiopia	2017	Districts	279	Mainly children	4%	Global malnutrition	Hepatitis A	Hygiene and water issues	Self-limited
Liberia	2017	Suburb of town	31	Young adults	42%		*Neisseria meningococcus* C	Clustering in time and space not explained	Self-limited
Cambodia	2012	Hospital catchment area of capital city	74	Children <3 y old	98%	Malnutrition	Enterovirus 71	Unexpected severity not explained	Self-limited
Cameroon	2017	Hospital catchment area in conflict zone	40	Children <5 y old	33%	Malnutrition	Visceral leishmaniasis	Yes, with identification of agent	Intervention started in 2020
Congo	2018	District	809	Adults	24%		Unknown	No	Unknown
Vietnam	2011	Villages	>200	All ages	16%	Malnutrition	Unknown	No	Unresolved by 2022
São Tomé and Príncipe	2016	Country	3000	Mainly adults	0%	Acquired immunodeficiency	Several agents	No	Intervention in 2018, resolved by 2020

Table 2. Classification of the 10 case studies according to the outcome of the outbreak investigation, country, syndrome, year of initial investigation and references

Category	Case study country	Syndrome	Year of investigation	References
Complete outcome	Angola	Neurological	2007	
	Nigeria	Neurological	2010	5–12
	DRC	Neurological	2014	13
	Ethiopia	Acute jaundice syndrome	2017	
	Cameroon	Fatal eruptive lesions and rash	2017	14
Incomplete outcome	Liberia	Severe gastrointestinal and neurological syndrome	2017	15,16
	Cambodia	Fatal respiratory and neurological syndrome—hand, foot and mouth disease	2012	17,18
Unsatisfactory outcome	Congo	Fatal febrile illness	2017	
	Vietnam	Fatal and severe skin conditions, multipunctate palmoplantar hyperkeratosis	2011	
	São Tomé and Príncipe	Necrotizing cellulitis	2016	19

Investigation time frames

It was not possible to ascertain an exact start date for most of the events, but the estimates available indicated that the delay between onset of the outbreak and an investigation being initiated ranged from 1 d (in Liberia) to several months, with a mean of 2.7 months and a median of 2.0 months. For the five case studies with a complete outcome, the duration of the investigation in four of the case studies varied from 1 to 5 months and in the other case study, Cameroon, it lasted >34 months with iterative periods of field work. For the two case studies with incomplete outcomes, Liberia and Cambodia, the investigations lasted for 15 d and 1 month, respectively. Of the three events with unsatisfactory outcomes, two were investigated iteratively over several years (São Tomé and Príncipe, Vietnam) and, to our knowledge, one was not monitored after an initial brief investigation lasting a few days (Congo).

Environmental surveys and case–control studies (CCSs)

Environmental surveys were conducted in five of the case study investigations and were considered a key factor in finding the aetiological agent or its source in three of the case studies, all of which had complete outcomes. In the other two outbreaks where environmental surveys were performed, one with an incomplete outcome and one with an unsatisfactory outcome, they did not play a role in identifying the agent or its source.

CCSs were conducted as part of six investigations (Angola, Liberia, São Tomé and Príncipe, DRC, Cameroon, Cambodia) and requested, though not performed, in a further two investigations (Ethiopia, Congo). The CCSs proved to be of limited utility in most of the investigations and potentially may have hindered some of them. In Cameroon, logistical constraints arising from instability in the area meant that only a clustered and small number of suspected cases could be enrolled in the CCS, limiting extrapolation of the findings. In the Angola case study, the CCS failed to correctly identify the causative agent and identified several indirect and erroneous risk factors. In the Liberia case study, the CCS erroneously appeared to indicate that consumption of some specific food products were risk factors for infection. It is likely that the findings were an artefact of the causative agent being spread among a group of people sharing a meal; however, the findings were initially interpreted as being indicative of a foodborne outbreak. In the DRC case study, the CCS was performed only after the correct causative agent had been identified, meaning that it had little impact on the trajectory of the investigation. In Cambodia, the CCS exploring the treatment modalities as a possible cause of the high CFR was inconclusive. As it is still unclear what causative agent was responsible for the São Tomé and Príncipe event, it is difficult to understand the relevance of the findings produced by the CCS.

Three investigations benefitted from a cross-sectional door-to-door survey, which helped to describe the geographic distribution of cases and establish baseline numbers (Angola), estimate the rate of increased mortality in households (Nigeria) and define the geographic spread and epidemiological features of the event (Vietnam).

Laboratory investigations

All 10 of the case studies involved laboratory testing and 8 case studies involved more than two rounds of laboratory testing. In nine of the events, laboratory support was acquired outside the affected country. In eight of these events, environmental samples, including soil, water, pharmaceuticals and food were tested, in addition to human biological samples.

Two distinct approaches to laboratory testing emerged across the case studies. One approach involved the use of targeted testing to confirm the presence of a particular causative agent suspected through clinical assessment, environmental investigation and interpretation of the descriptive epidemiology. The other approach involved identifying the causative agent by

subjecting human or environmental samples to either non-specific tests or tests that simultaneously test for a multitude of possible causative agents. Both approaches proved effective at times. 'Scattershot' testing appeared to offer significant advantages when investigating an outbreak of unknown aetiology where there was an atypical clinical presentation of the causative agent, as occurred in Liberia. In the case of chemical poisonings, laboratory investigations were most effective when the clinical syndrome pointed to a specific substance or group of substances.

In all 10 case studies, diagnostic testing revealed high burdens of other infectious or environmental disease-causing agents. There was also one incident of a false-positive result that nearly prematurely ended investigations. There were also two false-negative results, the impacts of which were mitigated through the testing of different sample types, the use of a variety of tests and the testing of severely ill patients.

The collection, storage and transport of samples often proved logistically challenging and were hampered by a lack of technical knowledge or adequate material, issues around interinstitutional coordination and significant resource constraints. Recurring issues included uncertainty around which samples to collect, poor quality of the samples, inadequate sample documentation, problems with packaging and storage during transport, difficulties getting official clearance for sample export, difficulties identifying an appropriate laboratory to perform the testing, the cost of specific laboratory tests and samples going missing at all stages of the testing process. A failure to communicate salient medical information and contextual indications that might guide laboratory investigations was reported as hampering testing efforts. Difficulty in linking laboratory test results to individual patients was widely reported as impairing the interpretation of laboratory findings.

Partnership and collaboration

All 10 of the case study investigations involved the WHO (represented by country office, regional office or headquarters) and at least one other transnational institution in addition to the national ministry of health. Nine of the case studies featured at least three transnational institutions and most involved more. The most common transnational institutions were Médecins Sans Frontières (MSF), the US Centers for Disease Control and Prevention (CDC) and Institut Pasteur. The CDC was involved in seven investigations either directly, via its national offices or via the WHO Global Outbreak Alert and Response Network. Institut Pasteur and MSF played important roles in five of the investigations.

Doctors or toxicologists outside the affected country were consulted in seven case studies through teleconferences and the circulation of photos, videos or case descriptions of patients. This consultation facilitated the identification of the agent or the syndrome in five case studies, in some cases by concluding that a chemical cause was unlikely, thus narrowing the focus. External collaborators assisted with the identification of suitable laboratories to undertake testing. This was particularly important for chemical analyses, as there is no established global network of toxicological laboratories.

Analysis of the cause of late recognition of the aetiology of the outbreak

Clinical characterization and development of a case definition

In five (half) of the case studies, the event was initially incorrectly attributed to a specific notifiable disease, namely, meningitis, malaria, hepatitis or Ebola. These early misdiagnoses were based on initial clinical assessment, location of the event, season and local epidemiology. For example, in Nigeria, meningitis was initially suspected, so fever was included in the first case definition despite many cases having been afebrile, as the true cause was lead poisoning.

Unusual or atypical clinical presentations were a recurring hinderance. For example, the unusually severe clinical pictures with high CFRs in the Ethiopia and Cambodia case studies initially appeared to contraindicate the correct diagnoses of hepatitis A and EV-71, respectively. In three further case studies (Angola, Liberia, Cameroon), the agent responsible for the outbreak was suspected early during the investigation but discarded as a result of perceived incompatibilities with the clinical picture or epidemiological features (compared with academic knowledge) or a lack of comprehensive knowledge of local disease incidence. In the Angola case study, bromide compounds were initially considered as a potential causative agent. They were discarded because the clinical picture did not correspond to any known descriptions of acute or chronic bromide poisoning. The unexpected clinical presentations were secondarily attributed to a range of factors, including co-infections (Ethiopia, Cameroon), the presence of comorbidities, malnutrition, and poor health status (Ethiopia), alcoholism (São Tomé and Príncipe, Liberia), the use of immunosuppressant therapies before admission (Cambodia), the genetics of the affected population (Cambodia, Vietnam) or the method of estimating CRF using only hospital-admitted patients in the denominator (Cambodia).

Epidemiological information

For two events with incomplete outcomes, Vietnam and Congo, the absence of systematic reporting of patient histories and thorough descriptive epidemiology hindered the exploration of possible routes of transmission and the generation of an adequate explanation for the event.

Two outbreaks presented with unusual epidemiological features. In Liberia, the apparent presentation of a point source outbreak following a social event triggered the search for a food poisoning–associated aetiological agent. Later, the outbreak was confirmed as a cluster of septicaemia of *Neisseria meningitidis* serogroup C, which is spread through human-to-human transmission. By the time the correct aetiological agent was identified, it was no longer possible to explore the transmission pattern of this unique outbreak of septicaemia of *N. meningitidis* serogroup C. Second, in Cameroon, the results of an entomological investigation published 1 y after the alert identified the agent of visceral leishmaniasis in sand flies captured in the area, a geographic focus of visceral leishmaniasis that was previously unknown. This triggered further epidemiological and laboratory investigations and allowed the identification of the source of the long-lasting outbreak for which leishmaniasis had already been suspected and discarded.

Laboratory information

Laboratory testing of samples identified the presence of numerous aetiological agents in all the case studies. In the Congo case study, four cases that tested positive for yellow fever at the field level were secondarily identified as false positives after a second testing; in the same case study, an incidental finding of a holoendemic infectious agent, *Plasmodium falciparum*, appears to have truncated the investigation.

Perceived risk and consequent prioritization of laboratory testing of highest-risk pathogens hindered investigation of the outbreak in Ethiopia. While hepatitis A virus was the most likely causative agent and was subsequently proven to be the correct causative agent with respect to the clinical and epidemiological picture, it was not requested for testing before the second round of samples was sent to the Dakar Pasteur Institute. Instead, initial laboratory investigations focused on testing for severe diseases such as yellow fever, Ebola virus disease and dengue fever.

Process of investigation

The process of identifying the correct causative agent was often iterative, requiring multiple forms of evidence gathering. The sequence of investigative activities varied between case studies and sometimes involved repeating the same investigative activity many times, such as laboratory testing, CCSs and environmental surveys.

Documentation of the investigations and their findings

Published accounts of previous investigations into similar events proved helpful in investigating an outbreak with an unusual clinical or epidemiological presentation in two of the case studies (Ethiopia, Angola). At the time of this writing, five of the case studies have been published in peer-reviewed journals. However, little is said about the challenges faced in the investigations. Accounts of the investigations appear in reports on institutional websites, in ProMed posts or as training or conference materials.

Discussion

We focused our efforts on identifying and describing the transnational responses to outbreaks that do not systematically generate public attention and that suffer from limited resources for investigation. These settings have proven to be possible sources of emerging diseases with pandemic potential, such as SARS in 2003[20] and the H1N1 swine-origin influenza pandemic in 2009.[21] An important criterion for selection of the 10 case studies was the availability of first-hand information on the investigations from public health professionals (most of them are co-authors of this study). We intended to use this information to evaluate the investigatory practices and the difficulties faced, as well as to gain insights into factors that favour or limit identification of aetiologies, sources or transmission patterns in remote settings. To our knowledge, best practices in the investigation of outbreaks of unknown aetiology in remote settings do not yet exist.

As a retrospective analysis of the investigations, there are some limitations. The accounts of the investigations are not all verified in official reports and might sometimes even contradict the official version. As some outbreaks occurred 10 y ago, there may be some gaps in the recall of the investigation process by informants and some descriptions may be incomplete.

It was difficult to clearly identify investigative methods universally favouring or limiting outbreak resolution because of the wide variation in the type, severity, time frame and scale of the events. However, some common challenges were identified: difficulty in generating an effective case definition; the iterative process of the investigative activities, with consequent resource needs; difficulty in integrating the social and epidemiological environments into the analysis; difficulties in obtaining timely and good-quality laboratory investigations; and multiple positive laboratory findings of variable relevance. Factors that facilitated the investigations were the use of remote expert support via video or pictures of clinical findings and collaboration between national and international institutions.

Interviewees reported that a major challenge during the investigation of outbreaks of unknown aetiology was obtaining accurate and complete descriptions of clinical signs and symptoms, patient history and complete epidemiology of the event. As with Silarug et al.[22] in Thailand, who described in the 1990s the association in one outbreak of influenza A with dengue fever, we highlight that secondary medical conditions, whatever the cause, play an important role in transforming the medical picture into one that is unexpected, atypical or confusing when compared with current knowledge and medical textbooks. One consequence of complex clinical presentations is that investigations are hindered as a result of an imprecise case definition. New disease trackers have been developed and can eventually serve to accelerate the identification of the disease[23]; however, the quality of the case description remains the key to the success of the disease tracker.

The complexity of the scenarios makes iterative investigations likely to first exclude more common causes or those easier to assess. However, investigations could have been better targeted in some cases had more precise and complete data been gathered first. Our study shows that when investigating outbreaks in complex settings, it is necessary to take account of the setting and context, including societal factors directly or indirectly affecting health. In investigating a cluster of cancer cases in the USA, Simpson et al.[24] recommended active listening, which seeks out peoples' perspectives, validates their concerns and engages them in the investigative process. In Cuba, the authors of an investigation into an outbreak of optic and peripheral neuropathies documented the myriad challenges in detecting, investigating and intervening in a syndromic problem in a complex setting of substantial social and economic transition, as was the situation in most of our case studies.[1] Guha-Sapir and Scales[25] declared that understanding the political dynamics of the outbreak setting is important to undertaking meaningful research and getting the results out in time (or at all).

In Ethiopia, Liberia and Cameroon, laboratory investigations focused on a variety of agents that were unlikely given clinical and epidemiological analysis. Cornejo et al.[26] reported how an unidentified cluster of infection in the Peruvian Amazon region was wrongly attributed using standard microbiological methods; the cause could be identified only by polymerase chain reaction using universal primers. In the DRC, the outbreak of neurological syndrome initially attributed to meningitis and

finally linked to falsified drugs emphasizes the importance of investigating atypical clinical presentations, of building precise and objective case definitions and the need for multidisciplinary approaches.[13]

The results of laboratory investigations in the DRC, Ethiopia, Cameroon, Vietnam and São Tomé and Príncipe reveal that more than one agent can be identified in patients' biological tests during investigations but are not always pertinent to the outbreak. This highlights the danger of deciding on the cause based on the first positive result. Several factors can contribute during an outbreak, or there may be more than one event occurring concurrently, leading to atypical epidemiology.

Based on analysis of the case studies, we propose the following recommendations to improve the process of investigating outbreak of unknown aetiology:

- Investigation
  - Organize a multidisciplinary team whose composition is adapted to the context and potential aetiologies. Suggested expertise includes epidemiologists; medical practitioners; laboratory specialists with material for taking and storing samples or, where feasible, a mobile laboratory; animal health specialist representative from a ministry of health; environmental specialist; and social science expert (anthropologist, behavioural science expert, sociologist).
  - Be aware and document the event that triggered the alert. Document what has happened since the alert. Many factors can modify the transmission mode of an agent, which can switch from unique source to person to person, or the reverse.
  - Create a line list of first cases with clinical characteristics and comorbidities, and including treatments received before presentation, which can serve as a test to build hypotheses regarding disease aetiology, especially when not successful.
  - Use existing case investigation forms and adapt them to the context (see WHO Outbreak Toolkit/Standardized data collection/T0 case report form).
  - When data have already been collected, conduct an audit of the data comparing them with medical fields or medical registers.
  - Propose precise, objective, operational, logical case definitions using available information for medical and laboratory findings, and limit these with time and place criteria. Determine the sensitivity and specificity required and re-evaluate as often as needed.
  - Be aware of the effects of comorbidities on atypical clinical presentations or the epidemiological picture and the possibility of false-positive and -negative laboratory results.
  - Review case definition and hypotheses as new information becomes available.
  - Collect, via key informants, cultural habits, societal structure, behaviours and information that can affect the transmission of an agent.
  - Actively seek local input into investigations via key informants to draw hypotheses, inform interpretation of the data and findings and discuss hypotheses for the cause, source or transmission.
  - Conduct additional studies, such as case–control, only if you can guarantee that they will be conducted with enough quality to allow interpretation of findings with confidence (watch size of the sample, selection of control, quality and reliability of answers).
- Proactive preparation for future outbreaks
  - Have modular questionnaires ready to adapt to the situation, covering medical, epidemiological and environmental issues.
  - Establish agreements/memoranda of understanding between institutions usually engaged in such investigation and with ministries of health in advance.
  - Establish links with specialist resources such as international clinical experts, environmental experts and reference laboratories, and have clear processes for using them.
  - Support (including with funding) outbreak investigation infrastructure, such as a field epidemiology training programme and rapid response teams that can facilitate complex investigations.
- Learn from past outbreaks
  - Publish descriptions of investigations of complex outbreaks, even when the cause is not determined, to alert the scientific community so that new knowledge from outbreaks can inform updates to medical texts.
  - Strengthen the WHO Emergency Monitoring System recording of follow-ups and outcomes to serve as an open international database of all outbreaks reported.

Conclusions

To support countries in addressing the investigation of outbreaks of unknown aetiology in remote and complex settings, the WHO Outbreak Toolkit project will use the observations gathered in this review to develop guidance and tools to improve the quality and timeliness for initial data collection and investigation of outbreaks of unknown aetiology. The toolkit hosts several tools: a recently published WHO manual for investigating outbreaks of possible chemical aetiology,[27] guidance for investigating clusters of respiratory disease of unknown aetiology and other syndromes[28] and a questionnaire for early investigation of outbreak of unknown aetiology,[29] among others.

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All authors contributed to revision of the manuscript and agree with the content.

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