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## Review Article

# Reverse microbial etiology: A research field for predicting and preventing emerging infectious diseases caused by an unknown microorganism

Jianguo Xu

State Key Laboratory of Infectious Disease Prevention and Control, National Institute for Communicable Disease Control and Prevention, Chinese Center for Disease Control and Prevention, Changping, Beijing 102206, PR China



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## ABSTRACT

To predict, detect, prepare for, and prevent potential emerging infectious diseases caused by unknown microorganisms in the future, we have proposed the research field of reverse microbial etiology. We isolate and classify unknown microorganisms and assess their ability to cause infection, an outbreak, or epidemic. We suggest a list of potential pathogens and propose a preparation, prevention, and control strategy to protect global health and global economy and to ensure global security.

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## 1. Introduction

An emerging infectious disease (EID) caused by an unknown microorganism poses a significant threat to global health, global economy, and global security by causing numerous deaths across cultural, political, and national boundaries as well as disproportionately high economic losses, exemplified by severe acute respiratory syndrome and Middle East respiratory syndrome coronavirus.<sup>1–3</sup> Delays in the detection of a causative pathogen or in the response—combined with increased global urbanization and connectivity—may result in social instability.<sup>4</sup>

Efforts to identify the origins and causative agents are necessary for diagnosis, treatment, limiting the spread, for control, or prevention of an EID caused by an unknown microorganism. When the microbial etiology of an outbreak caused by a new or unknown microorganism is considered definite, *Koch's postulates* must be satisfied.<sup>5</sup> Koch's postulates consist of the following requirements: 1) the organism must always be present in every case of the disease; 2) the organism must be isolated from a host containing the disease and grown in pure culture; 3) samples of the organism taken from pure culture must cause the same disease when inoculated into a healthy, susceptible animal in the laboratory; 4) the organism must be isolated from the inoculated animal and must be identified to be the same original organism first isolated from the original diseased host.<sup>5</sup> These principles have established the significance of laboratory culture of infectious agents. Koch's postulates are at the heart of traditional microbial etiology.

Microbial etiological investigations have been initiated after occurrence of an EID (Fig. 1).

Lately, because of concerns about global health, global economy, and global security, emphasis is being increasingly placed on predicting the occurrence of an EID caused by new or unknown microorganisms. If the potential EID can be predicted, it should be preventable when the public health measures are implemented. If the scientific alert can get ahead of the curve, then public health authorities may have a chance to respond to or even prevent the event. Therefore, to predict and prevent a potential EID caused by an unknown microorganism, we have proposed the research field of reverse microbiological etiology<sup>6</sup> (Fig. 1).

## 2. The postulates of reverse microbial etiology

Reverse microbial etiology involves the following postulates:

1. The unknown microorganism must be isolated, classified, and characterized. Alternatively, its complete genome should be sequenced, which can be used to assess its pathogenic or epidemic potential.
2. The pathogenicity of the newly classified or unknown microorganism should be assessed, including infection experiments on laboratory animals, cell lines, or tissues and analysis of the genome for putative virulence gene sequences.
3. The new or unknown microorganism should have the following abilities: 1) to infect a range of different animal species; 2) to spill over from animals to humans and then be transmissible from human to human; 3) to spread locally or internationally and get established in a new environment.

E-mail address: [xujianguo@icdc.cn](mailto:xujianguo@icdc.cn)

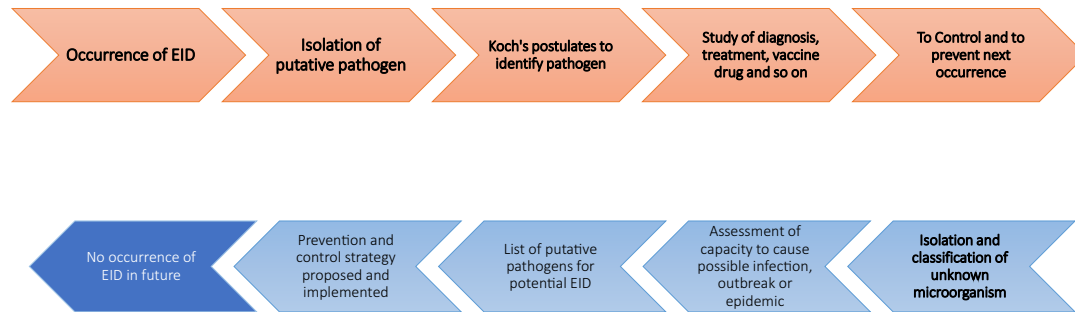


Fig. 1. Traditional microbial etiology and reverse microbial etiology.

4. Climate change and extreme weather events are potential future drivers of the emergence of an EID.
5. Changes in biodiversity, agriculture, land use, and social behavior are potential drivers of the disease emergence.
6. When the new or unknown microorganism is found to be a possible pathogen able to cause a local, nationwide, or international outbreak or epidemic, a detection, diagnosis, treatment, control, and prevention strategy should be formulated and implemented.

### 3. How many unknown potential pathogens have yet to be discovered?

The potential EID in the future is caused by an unknown or yet-to-be-discovered microorganism. Nevertheless, we know very little about the microbial world on Earth in terms of taxonomy and their traits that can cause a disease.

How many unknown viruses and bacteria are there on Earth? There are ~8.7 million eukaryotic species on Earth. If each eukaryotic species is associated with 10 species-specific viruses, then 87 million species of eukaryotic viruses is a good estimate.<sup>6</sup> One report suggests that there are approximately 1.67 million yet-to-be-discovered viral species of key zoonotic viral families in mammalian and bird hosts.<sup>7</sup> A metagenomic survey of 220 species in China identified 1445 novel RNA viruses, including many newly found genera, families, and possibly orders.<sup>8</sup> It has been suggested that the number of unknown species of viruses having a zoonotic potential ranges between 631,000 and 827,000.<sup>7</sup> Currently, the International Committee on Taxonomy of Viruses recognizes 4404 species of viruses affecting eukaryotes or prokaryotes.

By means of this scaling law combined with the lognormal model of biodiversity, Kenneth et al. predict that there are as many as 1 trillion ( $10^{12}$ ) microbial species on earth.<sup>9</sup> To date, only ~14,000 microbial species have been isolated and recognized. By the method of metataxonomics—integrating high-throughput sequencing of almost full-length small subunit ribosomal RNA (16S rRNA) gene amplicons in tandem with the operational phylogenetic unit (OPU) analysis—we found that there are 50 potential new species and 161 unknown new lineages of uncultured representatives in the feces of only nine vultures sampled in China.<sup>10</sup> We later showed that there are 256 potential new species and 103 potential higher taxa within known lineages, whereas 254 XYZ contain sequences not affiliated with any known family, and all the closest relatives are unclassified lineages of existing orders or classes in the feces of herbivorous Tibetan antelope inhabiting Qinghai-Tibet Plateau in China. Many new species of bacteria have been isolated from wild animals in China and characterized, including new animal pathogens.<sup>11</sup> It is unclear how many bacterial species on Earth have a pathogenic potential.

If we want to predict which microorganism can cause an EID next and where this may occur, the unknown microorganisms on

Earth must be isolated and classified or their genome should be sequenced and analyzed for the pathogenic or epidemic potential.

### 4. Discovery of new potentially pathogenic microorganisms in animals can alert clinicians to an EID in the future

Recently, a few new and potentially pathogenic microorganisms were isolated from animals first, and soon after, a corresponding human infection was diagnosed. A novel arenavirus designated as Wenzhou virus was isolated from five species of rats and one species of shrews in Wenzhou city of Zhejiang Province in China. A pathogenic potential was suggested, but not confirmed initially.<sup>12</sup> It was then found that some patients in southeast Asian countries with respiratory symptoms tested positive for the Wenzhou virus. The virus was hypothesized to be spreading more widely among humans in Asia.<sup>13</sup> The microorganism *Anaplasma capra* was first isolated from goats in China. The corresponding human infections were then identified in northern China, where the risk of exposure to novel tick-borne pathogens is higher.<sup>14</sup>

### 5. Studying the microorganisms in wild animals alerted clinicians to possible EIDs in China

In the period from 2013 to 2017, a group of scientists in China studied the microorganisms carried by wild animals such as ticks, mosquitoes, rats, bats, and marmots. Then, the scientists issued an alert about potential EIDs that may strike in the future in China.

1. West Nile virus (WNV) was isolated from *Culex pipiens pipiens* mosquitoes in the Kashi Region, Xinjiang, China. Human encephalitis previously caused by WNV in Xinjiang, China, was then confirmed; originally, these cases were misdiagnosed as infection with Japanese encephalitis virus.<sup>15</sup>
2. Zika virus was isolated from *Culex quinquefasciatus* collected in southern China in 2016; this mosquito can help replicate Zika virus and spread it to other animals through bites.<sup>16</sup>
3. SARS-related coronavirus (SARSr-CoV) has been isolated from multiple species of horseshoe bats in Yunnan Province, China. This finding highlights the necessity of preparedness for future emergence of SARS-like diseases in China.<sup>17</sup>
4. *Rickettsia sibirica* subspecies *sibirica* BJ-90, a variant of *R. sibirica*, was first isolated from *Dermacentor sinicus* on the campus of China CDC in 1990 and was recognized as a potential pathogen. The corresponding cases of severe human infection have been diagnosed in Mudanjiang Forestry Central Hospital in Mudanjiang, China.<sup>18</sup>
5. *Candidatus Rickettsia tarasevichiae* was initially detected in *Ixodes persulcatus* ticks in Russia in 2003.<sup>19</sup> The human infections were confirmed in China in 2012.<sup>20</sup>
6. The species of *Borrelia burgdorferi*, to be precise a genospecies, has been detected in ticks and rodents in China previ-

ously. The human infection was confirmed in 2010 and 2011 in Heilongjiang Province of northeastern China.<sup>21</sup>

7. *Babesia venatorum* has been identified in only a few asplenic men and a child previously. Forty-eight human cases of infection in northeast China were reported in 2014.<sup>22</sup>
8. *Rickettsia raoultii* was first detected in ticks in the former Soviet Union in 1999. A human infection was reported in France in 2009. Human infections were confirmed in China in 2012.<sup>23</sup>
9. *Borrelia miyamotoi* was first identified in ticks and rodents in Japan in 1994. A human infection was confirmed in Russia in 2011. Human infections were reported in China in 2018.<sup>24</sup>
10. A novel spotted fever group of *Rickettsia*, designated as *Rickettsia* sp. XY99, was identified in central China. Human infection was diagnosed in 2015.<sup>25</sup>
11. A novel hepatovirus tentatively named *Marmota himalayana* hepatovirus (MHHAV) was isolated from the wild animal *Marmota himalayana* in China. Phylogenetic analysis of MHHAV and primate hepatoviruses has led to the most recent common ancestor ~1000 years ago. Nevertheless, no human infection has been identified so far.<sup>26</sup>
12. A new subtype of tick-borne encephalitis virus (TBEV) has been discovered in the wild rodent *Marmota himalayana* on Qinghai-Tibet Plateau in China and proposed to be a Himalayan subtype (Him-TBEV). Phylogenetic analysis suggests that Him-TBEV diverged from other subtypes of the eastern TBEV group ~2469 years ago. Public health significance should be evaluated carefully.

#### Conflict of interest

The author declared that he have no conflicts of interest to this work.

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