



## REVIEW


### REVIEW

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# The Mengla virus (*Filoviridae: Dianlovirus*)

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### Abstract

**Introduction.** Filoviruses associated with various species of pteropodid bats (*Chiroptera: Pteropodidae*) are traditionally regarded as potential causative agents of hemorrhagic fevers with epidemic potential. The known agents of Ebola and Marburg fevers periodically cause sporadic cases and epidemic outbreaks in African countries. Recent discoveries of novel filoviruses associated with pteropodid bats in South and Southeast Asia highlight the necessity to investigate their genetic diversity and pathogenic potential.

The **aim** of this study was to investigate the genetic diversity and pathogenic potential of new filoviruses associated with bats, based on literature data.

**Materials and methods.** This review is based on an analysis of published literature describing the detection and molecular characterization of novel filoviruses identified in different geographic regions, with a particular focus on filoviruses associated with pteropodid bats in South and Southeast Asia. The analyzed studies include data on virus discovery, genome organization, taxonomic classification, and experimental assessment of biological properties.

**Results.** Several novel filoviruses have been identified by metagenomic RNA sequencing of tissues from pteropodid bats captured in South and Southeast Asia. Among them, Mengla virus was detected in tissues of pteropodid bats (*Rousettus* spp.) captured in Mengla County, Yunnan Province, People's Republic of China. Owing to a high level of genetic divergence, Mengla virus was classified as a representative of a new genus, *Dianlovirus*, within the family *Filoviridae*. Although a live isolate of Mengla virus has not yet been obtained, experimental studies using chimeric minigenome systems and virus-like particles suggest that the virus may exhibit tropism for tissues of various vertebrate hosts, including humans.

**Conclusion.** Members of the family *Filoviridae* are widely distributed within the geographic range of their natural reservoir—pteropodid bats—across South and Southeast Asia, including viruses evolutionarily related to Ebola and Marburg viruses. Although human disease caused by Mengla virus and other recently discovered filoviruses has not been documented, the potential for cross-species transmission and the emergence of novel filovirus infections in endemic regions remains.

**Keywords:** *Mengla virus; filoviruses; sequencing of nucleic acids; sequence of genome; bats; natural reservoir of filoviruses*

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## НАУЧНЫЙ ОБЗОР

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## Вирус Менгла (*Filoviridae: Dianlovirus*)

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### Резюме

**Введение.** Филовирусы, ассоциированные с различными видами крыланов (*Chiroptera: Pteropodidae*), традиционно рассматриваются как потенциальные возбудители геморрагических лихорадок с эпидемическим потенциалом. Возбудители лихорадок Эбола и Марбург периодически вызывают спорадические случаи и эпидемические вспышки в странах Африки. Открытие в последние годы новых филовирусов, связанных с рукокрылыми в Южной и Юго-Восточной Азии, обуславливает необходимость изучения их генетического разнообразия и патогенного потенциала.

**Цель работы.** Изучение генетического разнообразия и патогенного потенциала новых филовирусов, ассоциированных с рукокрылыми, на основе данных литературы.

**Материалы и методы.** Обзор основан на анализе публикаций, посвященных выявлению и молекулярной характеристике новых филовирусов, обнаруженных в разных географических регионах, с акцентом на филовирусы, ассоциированные с крыланами, в Южной и Юго-Восточной Азии. Проанализированы сведения об обнаружении вирусов, организации их геномов, таксономической классификации и экспериментальной оценке биологических свойств.

**Результаты.** В последние годы выявлен ряд новых филовирусов в тканях крыланов, отловленных в разных регионах Южной и Юго-Восточной Азии. Вирус Менгла был обнаружен в тканях печени летучих собак (*Rousettus* spp.), отловленных в округе Менгла провинции Юньнань Китайской Народной Республики. В связи с высоким уровнем генетической дивергенции вирус Менгла отнесен к новому роду *Dianlovirus* в составе семейства *Filoviridae*. Несмотря на отсутствие выделенного живого изолята, экспериментальные исследования с использованием химерных минигеномных систем и вирусоподобных частиц указывают на возможный тропизм вируса Менгла к тканям различных позвоночных животных, включая человека. Заключение. Представители семейства *Filoviridae* широко распространены в пределах ареала их природного резервуара крыланов (семейство *Pteropodidae*) и, возможно, летучих мышей на территории Южной и Юго-Восточной Азии, включая вирусы, эволюционно родственные вирусам Эбола и Марбург. Несмотря на отсутствие документированных случаев заболеваний человека, вызванных вирусом Менгла и другими недавно выявленными филовирусами, сохраняется вероятность межвидовой передачи и возникновения новых филовирусных инфекций в эндемичных регионах.

**Ключевые слова:** вирус Менгла; филовирус; секвенирование нуклеиновых кислот; последовательность генома; рукокрылые; резервуар филовирусов

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**Финансирование.** Авторы заявляют об отсутствии внешнего финансирования при проведении исследования.

**Конфликт интересов.** Авторы декларируют отсутствие явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

### Introduction

Diseases associated with Marburg and Ebola filoviruses (family *Filoviridae*) pose a serious threat to public health due to their high mortality rate and ability to cause epidemic outbreaks [1, 2]. Since the discovery of the Marburg virus in 1967 and the Ebola virus in 1976, outbreaks of hemorrhagic fever have been repeatedly reported, mainly on the African continent, with cases of the virus being brought to Europe and the United States by infected medical workers [1–3].

The family *Filoviridae* was originally organized on the basis of combining genera corresponding to Marburg (*Marburgvirus*) and Ebola (*Ebolavirus*) viruses, based on common morphological characteristics and genome type and structure (single-stranded negative-sense RNA – ss(–)RNA). Based on these same genomic characteristics, the family was included in the order *Mononegavirales*,

one of four orders (taxon above the family) that existed at that time. Currently, due to the development of molecular genetic and evolutionary approaches, the taxonomy of the family has been significantly expanded and refined and is as follows: *Riboviria: Orthornavirae: Negarnaviricota: Monjiviricetes: Mononegavirales: Filoviridae*. The genus *Orthomarburgvirus* (formerly *Marburgvirus*) includes two species, while the genus *Orthoebolavirus* (formerly *Ebolavirus*) now includes six species (Table 1). Most Marburg and Ebola viruses appear to be ecologically associated with various species of fruit bats (*Chiroptera: Pteropodidae*) living in Africa and Asia. An exception is the Bombalivirus (*Orthoebolavirus bombaliense, Bombalivirus*), whose RNA was detected in bulldog bats (*Mopscondylurus*) in Sierra Leone in 2018. Later, a new genus, *Cuevavirus*, was assigned to the family *Filoviridae*, represented by the Lloviu virus (*Lloviuvirus*), which was

detected in samples from a colony of common bent-wing bats (*Miniopterus schreibersii*) that died in 2002 in Spain [3–7]. Thus, filoviruses may be ecologically associated not only with different species of fruit bats (fruit-eating flying foxes or flying dogs), but certain species of bats may also be involved in their circulation.

Currently, the *Filoviridae* family includes nine genera of viruses found in Africa and Asia, of which only Ebola and Marburg viruses have been isolated from humans. RNA from various filoviruses has been detected in eight species of bats living in different regions of Africa, Asia, and Europe (Table 1) [3, 6, 8–10]. Furthermore, virus-specific antibodies have been detected in eight different species of bats [9–15]. At the same time, only one live filovirus isolate (Marburg virus associated with an outbreak of hemorrhagic fever among miners in Uganda in 2007) has been isolated from these animals [16]. In this regard, the question of the natural reservoir of filoviruses in nature requires further research.

### Filoviruses in South and Southeast Asia

In Asia, the circulation of filoviruses was first described based on the detection of antibodies to Ebola virus nucleoproteins (NP) and glycoproteins (GP) in fruit bats (*Rousettus amplexicaudatus*) in the Philippines in 2010, while samples from five other bat species and ten bat species tested were negative [13]. Antibodies to the Reston virus and its RNA were also detected in fruit bats, pigs, and farm workers in Bangladesh [10]. In the People's Republic of China (PRC), antibodies reacting with the Ebola virus antigen were detected in several species of bats in 2012 [17].

Studies on the detection of filoviruses in various species of bats, carried out using metagenomic sequencing or universal primers in the PRC, led to the description of previously unknown filoviruses that show significant divergence from known species and genera circulating in Africa. The identified viruses were fully sequenced and formed three new genera within the family *Filoviridae*: *Dianlovirus*, *Striavirus*, and *Thamnovirus*, whose prototypical representatives are the Mengla, Xilang, and Hungjia viruses, respectively (Table 2) [3].

### Mengla virus: detection and molecular genetic characterization

The Mengla virus was detected during screening studies of organ samples (intestines, lungs, and brain) obtained from bats *Rousettus leschenaultia* (*Chiroptera: Pteropodidae*) caught in Yunnan Province, China, in 2013. The virus was detected by metagenomic analysis and using species-specific universal primers to conserved regions of the genome [19]. The partial genomic sequences obtained were similar to various genes of the Lloviu virus, Sudan virus, and Tai forest virus<sup>1</sup>. Attempts to isolate the biologically active virus in Vero E6 cells were unsuccessful. As a result, the genome of the new virus was amplified and almost completely sequenced. The results of genetic analysis

**Table 1.** The modern taxonomy of the family *Filoviridae*

**Таблица 1.** Современная таксономия семейства *Filoviridae*

Genus Род	Species Вид	Prototype virus Прототипный вирус
<i>Cuevavirus</i>	<i>Cuevavirus lloviuense</i>	Lloviuvirus [LLOV]
<i>Dianlovirus</i>	<i>Dianlovirus dehongense</i>	Déhongvirus [DEHV]
	<i>Dianlovirus menglaense</i>	Menglavirus [MLAV]
<i>Loebivirus</i>	<i>Loebivirus percae</i>	Lötschbergvirus [LTBV]
<i>Oblavirus</i>	<i>Oblavirus percae</i>	Oberlandvirus [OBLV]
<i>Orthoebolavirus</i>	<i>Orthoebolavirus bombaliense</i>	Bombalivirus [BOMV]
	<i>Orthoebolavirus bundibugyoense</i>	Bundibugyoivirus [BDBV]
	<i>Orthoebolavirus restonense</i>	Restonvirus [RESTV]
	<i>Orthoebolavirus sudanense</i>	Sudanvirus [SUDV]
<i>Orthoebolavirus</i>	<i>Orthoebolavirus taiense</i>	Tai Forestvirus [TAFV]
	<i>Orthoebolavirus zairense</i>	Ebolavirus [EBOV]
	<i>Orthomareburgvirus marburgense</i>	Marburgvirus [MARV] и Ravnvirus [RAVV]
<i>Striavirus</i>	<i>Striavirus antennarii</i>	Xilangvirus [XILV]
<i>Tapjovirus</i>	<i>Tapjovirus bothropis</i>	Tapajósvirus [TAPV]
<i>Thamnovirus</i>	<i>Thamnovirus kanderense</i>	Kandervirus [KNDV]
	<i>Thamnovirus percae</i>	Fiwivirus [FIWIV]
	<i>Thamnovirus thamnaconi</i>	Huángjiāovirus [HUJV]

(32–54% similarity when comparing complete genomes) allowed the Mengla virus to be isolated into a separate genus, named *Dianlovirus*, within the family *Filoviridae*. In dendrograms constructed on the basis of various proteins or complete genomes, the Mengla virus (genus *Dianlovirus*) forms a separate branch located between the viruses of the genera *Orthomareburgvirus* and *Orthoebolavirus* [18–20].

The structure of the Mengla virus genome is characteristic of filoviruses. It has a negative-polarity RNA genome (ss(–)RNA) with the typical filovirus gene arrangement and composition: *NP–VP35–VP40–GP–VP30–VP24–L*. However, the Mengla virus has specific differences in the number of overlapping genes and a divergent sequence structure of the transcriptional terminal sequence. Unlike Ebola and

<sup>1</sup>Available at: <http://wwwnc.cdc.gov/EID/article/21/9/15-0260-Technapp1.pdf>

**Table 2.** Time, place and source of isolation or detection of different filoviruses

**Таблица 2.** Год, место и источник выделения или обнаружения различных филовирусов

Genus Род	Virus Вирус	Year of detection Год выявления	Country of first isolation of pathogen Страна, в которой впервые был обнаружен возбудитель	Isolation source of pathogen Источник выделения возбудителя
<i>Orthomarburgvirus</i>	<b>Marburg</b> <b>Марбург</b>	1967	Germany Германия*	
	<b>Ravn</b>	2007	Uganda Уганда	Human Человек
	<b>Zaire</b> <b>Эбола-Заир</b>	1976	Zaire (DRC) Заир (ДР Конго)	
<i>Orthoebolavirus</i>	<b>Эбола-Судан</b> <b>Sudan</b>	1976	Sudan Судан	
	Reston Эбола-Рестон	1989	USA США*	Monkeys Низшие приматы
	<b>Tai Forest</b> <b>Леса Таи</b>	1994	Uganda Уганда	Human Человек
	<b>Bundibugyo</b> <b>Бундибяго</b>	2007	Uganda Уганда	
	Vombali Бомбали	2018	Sierra Leone Сьерра-Леоне	
<i>Cuevavirus</i>	Lloviu Ллови	2003	Spain Испания	
<i>Dianlovirus</i>	Mengla Менгла	2015		Bats Рукокрылые
<i>Striavirus</i>	Xilang Ксиланг	2017	China КНР	
<i>Thamnovirus</i>	Huangjiao Хунджиао	2017		

**Note.** Ebolaviruses and Marburgviruses causing hemorrhagic fever in humans are highlighted in bold [18]; \* – imported cases in regions where the pathogen is not endemic.

**Примечание.** Эболавирусы и марбургвирусы, вызывающие геморрагические лихорадки человека, выделены полужирным шрифтом [18]; \* – завозные случаи в эндемичных по возбудителю регионах.

Lloviu viruses, the GP gene of the Mengla virus encodes only one GP [18].

Despite the absence of a live isolate, it has been shown that the Mengla virus is replication-competent in minigenomic systems containing the leader and trailer sequences of Ebola and Marburg viruses [20]. The GP protein of the Mengla virus is capable of transducing human, primate, dog, hamster, and bat cells, indicating broad cellular tropism and the potential for interspecies transmission [21]. Like other filoviruses, the Mengla virus uses the Neumann–Pick protein (NPC1) as a key receptor for cell entry [20, 22, 23].

Further monitoring studies of materials collected from various species of bats in 2009 and 2015 showed the widespread distribution of detected filoviruses in China (samples were collected at locations more than 200 km apart). In samples from bats of the *Eonycteris spelaea* and *Rousettus* sp. species, quantitative polymerase chain reaction (qPCR) was used to determine the tissue tropism of this group of filoviruses. Most often and in the highest titer, the Mengla virus and related viruses were detected

in lung tissue samples. Viral RNA was also detected in blood samples (2 samples) and in other organs and tissues of animals. Meanwhile, the virus titer (based on PCR) in lungs was 1–3 orders of magnitude higher than in other organs (liver, kidneys, spleen, intestines, heart, blood). The results obtained indicate that the lungs are the preferred target organ for the Mengla virus (**Table 3**).

### Conclusion

Thus, data obtained in recent years indicate significant genetic diversity and widespread distribution of filoviruses among flying foxes (*Chiroptera: Pteropodidae*) in different regions of the world, including South and Southeast Asia. The discovery of new genera of the family *Filoviridae*, including *Orthodianlovirus*, highlights the necessity for further research into the biological properties and evolutionary relationships of these viruses. Although flying foxes and possibly some bat species are the natural reservoirs of filoviruses, this group of viruses has a high potential for interspecies transmission to other mammalian species, including humans. The Mengla

**Table 3.** The results of detection of RNA and filovirus antigens in bats of different species captured in Province Yunnan, China in 2009 and 2015 by different modifications of PCR, ELISA and immunoblot analysis [17, 21]

**Таблица 3.** Результаты выявления РНК и антигена филловирусов у различных видов рукокрылых, пойманных в провинции Юньнань, КНР в 2009 и 2015 гг. с помощью различных модификаций ПЦР, иммуноферментного анализа (ИФА) и иммуноблота [17, 21]

Time of detection (year, month) Год, месяц выявления	Place of bat capture Место отлова летучих мышей	Bat species Вид рукокрылых	Detection of filovirus RNA Выявление РНК филловирусов		Reveal of specific antibodies against Ebola virus (Frequency of reveal) Выявление специфических антител к вирусу Эбола (частота выявления в пробах)					
			RT-qPCR ОТ-ПЦР-ПВ	qPCR qПЦР	ELISA ИФА		Immunoblot Иммуноблот			
					Zaire Заир	Reston Рестон	Zaire Заир	Reston Рестон		
2009 Ноябрь November	Jinhong, Xishuanbanna County г. Джинхонг, округ Сишунаньбанна	<i>Myotis ricketti</i> <i>Eonycteris spelaea</i> and <i>Rousettus</i> sp.	0/27		1/27					
2015 Декабрь December	Mengla County Округ Менгла	<i>Asellus stoliczkanus</i> <i>Eonycteris spelaea</i> and <i>Rousettus</i> sp.	10/43	5/43	6/43		2/43			
			5/42	10/42	0/15		14/25	7/25	11/25	4/25

**Note.** qPCR – quantitative polymerase chain reaction; RT-qPCR – real-time reverse transcription polymerase chain reaction.

**Примечание.** qПЦР – количественная полимеразная цепная реакция; ОТ-ПЦР-ПВ – полимеразная цепная реакция с обратной транскрипцией в режиме реального времени.

virus demonstrates tropism and the ability to accumulate in the lung tissue of flying squirrels, which contributes to the emergence of variants capable of respiratory transmission. The species of flying foxes surveyed in China are frugivorous and may also potentially contribute to the contamination of fruit and lead to the infection of animals and humans that come into contact with them. In this regard, it is particularly important to monitor filoviruses in regions geographically close to the Russian Federation, taking into account the migratory activity of bats and the potential ability of new filoviruses to transmit between species to other mammals.

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