



# OneBAT Training on Bat Sanitary Surveillance

20-21 May 2026 | Padova (Italy)

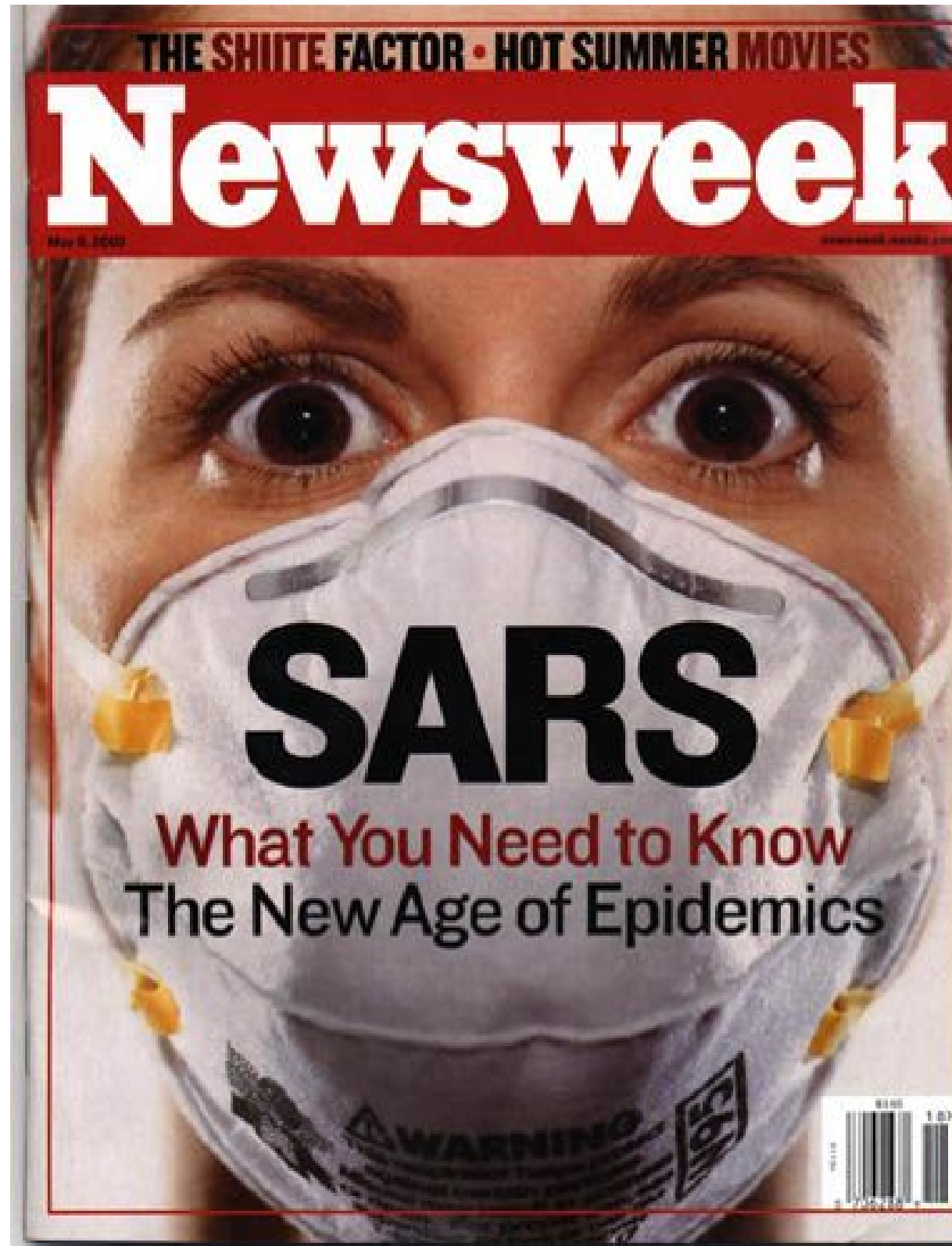
## Ecology of bat viruses

*Stefania Leopardi*



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Severe acute respiratory syndrome coronavirus-like virus in Chinese horseshoe bats

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Communicated by Lap-Chee Tsui, University of Hong Kong, Hong Kong Special Administrative Region, China, August 9, 2005 (received for review June 22, 2005)

Although the finding of severe acute respiratory syndrome coronavirus (SARS-CoV) in caged palm civets from live animal markets in China has provided evidence for interspecies transmission in the genesis of the SARS epidemic, subsequent studies suggested that the civet may have served only as an amplification host for SARS-CoV. In a surveillance study for CoV in noncaged animals from the wild areas of the Hong Kong Special Administration Region, we identified a CoV closely related to SARS-CoV (bat SARS-CoV) from 23 (39%) of 59 anal swabs of wild Chinese horseshoe bats (*Rhinolophus sinicus*) by using RT-PCR. Sequencing and analysis of three bat SARS-CoV genomes from samples collected at different dates showed that bat SARS-CoV is closely related to SARS-CoV from humans and civets. Phylogenetic analysis showed that bat SARS-CoV formed a distinct cluster with SARS-CoV as group 2b CoV, distantly related to known group 2 CoV. Most differences between the bat SARS-CoV and SARS-CoV genomes were observed in the spike genes, ORF 3 and ORF 8, which are the regions where most variations also were observed between human and civet SARS-CoV genomes. In addition, the presence of a 29-bp insertion in ORF 8 of bat SARS-CoV genome, not in most human SARS-CoV genomes, suggests that it has a common ancestor with civet SARS-CoV. Antibody against recombinant bat SARS-CoV nucleocapsid protein was detected in 84% of Chinese horseshoe bats by using an enzyme immunoassay. Neutralizing antibody to human SARS-CoV also was detected in bats with lower viral loads. Precautions should be exercised in the handling of these animals.

Coronaviruses (CoV) are found in a wide variety of animals in which they can cause respiratory, enteric, hepatic, and neurological diseases of varying severity. As a result of the unique mechanism of viral replication, CoV have a high frequency of recombination (1). Their tendency for recombination and high mutation rates may allow them to adapt to new hosts and ecological niches. Among CoV that infect humans, including human CoV 229E (HCoV-229E), human CoV OC43 (HCoV-OC43), severe acute respiratory syndrome CoV (SARS-CoV), human CoV NL63 (HCoV-NL63), and CoV HKU1 (CoV-HKU1), SARS-CoV causes the most severe disease, with >700 fatalities reported since the SARS epidemic in 2003 (2–8).

The isolation of SARS-CoV from caged animals, including Himalayan palm civets and a raccoon dog, from wild live markets in mainland China suggested that these animals are the reservoir for the origin of the SARS epidemic (9). However, subsequent studies suggested that the civet may have served only as an amplification host for SARS-CoV and provided the environment for major genetic variations permitting efficient animal-to-human and human-to-human transmissions (10–13). Because civets are often mixed with different species in overcrowded conditions at markets, we conducted a surveillance study for CoV in noncaged animals from the wild areas of the Hong Kong Special Administrative Region (HKSAR). In this report, we describe the identification and molecular characterization of a SARS-CoV-related virus from Chinese horseshoe bats in Hong

Kong and propose that this virus be named bat SARS-CoV (bat-SARS-CoV).

Methods

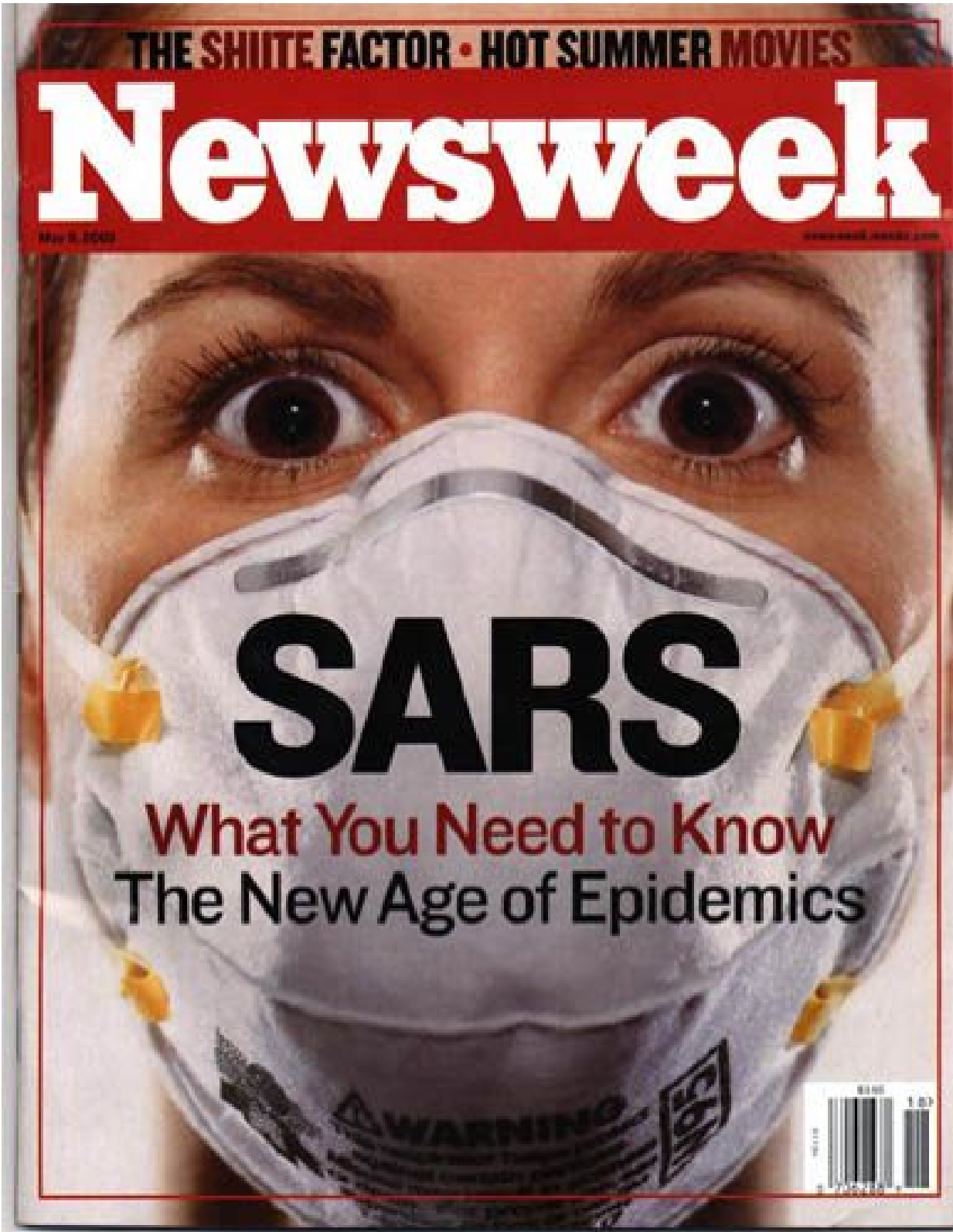
**Wild Animal Surveillance and Sample Collection.** The study was approved by the HKSAR's Department of Agriculture, Fisheries, and Conservation and the University of Hong Kong Committee on the Use of Live Animals in Teaching and Research. From summer 2004 to spring 2005, 127 bats, 60 rodents, and 20 monkeys from 11 locations in the HKSAR were captured (Fig. 1a and Table 2), which is published as supporting information on the PNAS web site). Nasopharyngeal and anal swabs and blood samples were collected by a veterinary surgeon. Swabs were taken with sterile swabs and kept in viral transport medium at 4°C before processing (14). Where possible, blood was collected for serological studies.

**RNA Extraction, RT-PCR, and DNA Sequencing.** Nasopharyngeal and anal swabs were tested for CoV RNA by RT-PCR. Viral RNA extraction was performed by using the QIAamp viral RNA mini kit (Qiagen, Valencia, CA) according to the manufacturer's instructions. cDNA was generated by using random hexamers and the SuperScript II kit (Invitrogen) as described in ref. 7. CoV screening was performed by using conserved primers (5'-GGTTGGGACTATCC1AAGTGTGA-3' and 5'-CCAT-CATCAGATAGATCATCATA-3') targeted to a 440-bp fragment of the pol gene as described in ref. 7. Standard precautions were taken to avoid PCR contamination, and no false-positive was observed in negative controls. The sequences of the PCR products were compared with known sequences of the pol genes of CoV in GenBank.

**Viral Cultures.** Attempts to isolate bat-SARS-CoV were made by inoculating RT-PCR-positive specimens to FRhK-4, HRT-18G, Huh-7, Vero E6, C6 /36 and Caco-2 cells, and chicken embryonated eggs. Viral replication was detected by observation for cytopathic effects and quantitative RT-PCR described below.

**Complete Genome Sequencing and Genome Analysis.** The complete genome of bat-SARS-CoV was sequenced by using RNA extracted from three anal swabs from three bats (B24, B41, and B43) as template. RNA was converted to cDNA by a combined random-priming and oligo(dT)-priming strategy described in ref. 7. A total of 63 sets of primers, available on request, were used for PCR. The 5' end of the viral genome was confirmed by rapid

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2002 SARS



Severe acute respiratory :  
virus in Chinese horsesho

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Communicated by Lap-Chee Tsui, University of Hong Kong, Hong Kong Special  
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1976 – 2024 Ebola

## Severe acute respiratory syndrome coronavirus-like virus in Chinese horseshoe bats

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**Abbreviations:** CoV, coronavirus; HCoV, human CoV; RT-PCR, reverse transcription-PCR; SARS, severe acute respiratory syndrome; HKSAR, Hong Kong Special Administrative Region; a, spike; N, nucleocapsid; E, envelope; immunosay, IELISA; angiotensin converting enzyme 2, ACE2. Data deposition: The sequences reported in this paper have been deposited in the GenBank database (accession nos. DQ301801, DQ301802, and DQ301803). H.K.P.L., P.C.Y.W., and K.Y.Y. contributed equally to this work. To whom correspondence should be addressed: E-mail: kyyuen@hku.hk. © 2005 by The National Academy of Sciences of the USA.

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in mainland China suggested that these animals are the reservoir for the origin of the SARS epidemic (9). However, subsequent studies suggested that the civet may have served only as an amplification host for SARS-CoV and provided the environment for minor genetic variations permitting efficient animal-to-human transmission (10). In a subsequent study, 100 civets were sampled and 10 different species in 100 roadside conditions at markets, we conducted a surveillance study for CoV in noncaged animals from the wild areas of the Hong Kong Special Administrative Region (HKSAR). In this report, we describe the identification and molecular characterization of a SARS-CoV-related virus from Chinese horseshoe bats in Hong

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## BRIEF COMMUNICATIONS

... and the search goes on...

### Fruit bats as reservoirs of Ebola virus

Bat species eaten by people in central Africa show evidence of symptomless Ebola infection.

The first recorded human outbreak of Ebola virus was in 1976, but the wild reservoir of this virus is still unknown<sup>1</sup>. Here we test for Ebola in more than a thousand small vertebrates that were collected during Ebola outbreaks in humans and great apes between 2001 and 2003 in Gabon and the Republic of the Congo. We find evidence of asymptomatic infection by Ebola virus in three species of fruit bat, indicating that these animals may be acting as a reservoir for this deadly virus.

Human Ebola outbreaks that occurred between 2001 and 2005 in Gabon and the Republic of the Congo were linked to concurrent outbreaks in du, deas and local gorilla and chimpanzee populations<sup>2,3</sup>. To identify the viral reservoir, we undertook three trapping expeditions in areas close to infected gorilla and chimpanzee carcasses, just after their discovery (Fig. 1a). In total, 1,039 animals were captured, including 679 bats, 222 birds and 129 small terrestrial vertebrates, and were tested for evidence of infection by Ebola virus (for details, see supplementary information).

Of the infected animals identified during these field collections, immunoglobulin G (IgG) specific for Ebola virus was detected in serum from three different bat species (4 of 17 *Hypsignathus monstrosus*, 8 of 117 *Eidolon helvum* and 4 of 58 *Myotis torquatus*). Two of the principal organs targeted by Ebola virus are the liver and spleen<sup>4</sup>. Viral nucleotide sequences were detected in these organs in other bats from the same populations (1 of 21, 5 of 117 and 4 of 141, respectively). No viral RNA was detected in kidney, heart or lung in these animals after amplification by polymerase chain reaction (PCR) and no viral nucleotide sequences were revealed in any of the other animal species tested.

Nucleotide sequence analysis of purified PCR products identified seven different fragments amplified from the 13 PCR-positive animals, all clustering phylogenetically within the Zaire clade (Fig. 1b). The fragments differed not only from one collection to another, but also within a given collection, among the three bat species, and within a given species. The need to use nested PCR indicated that the viral RNA load in tissues was extremely low, which probably explains why we failed to isolate the virus itself.

Surprisingly, none of the IgG-positive animals was PCR-positive, and none of the PCR-positive animals was IgG-positive. This may

be because PCR-positive bats were recently infected and were tested before they developed a detectable immune response. Alternatively, it could be that differences in the virulence of Ebola virus strains led to different immunological responsiveness and viral replication patterns. Of the bat species collected at Mbooro in February 2003, 7 of 31 (22.6%) and 10 of 10 (100%) were PCR-positive and IgG-positive, respectively, but five months later the corresponding results were 4 of 184 (2.2%) and 2 of 160 (7.5%). These opposite trends in the PCR and serological results are consistent with the first hypothesis.

Each of the three bat species has a broad geographical range that includes regions of Africa where human Ebola outbreaks occur (Fig. 1c). Our findings support results of previous investigations that identify bats as candidate reservoirs for Ebola and Marburg viruses<sup>5,6</sup>, and as reservoirs for the virus families *Morboviridae* and *Rhabdoviridae*<sup>7,8</sup>, which are genetically related to Ebola.

Mortality among great apes from Ebola infection can increase during the dry seasons when fruit is scarce in the forest — conditions that foster contact between animals as they compete for food. Immune function in bats also changes during these periods<sup>9</sup>, for example as a result of food scarcity or pregnancy, which would favour viral replication and — aided by aggressive interactions — increase infection among great apes. These factors may contribute to the episodic nature of Ebola outbreaks.

Although other bat and animal species may also act as Ebola virus reservoirs, insight into

Figure 1 | Fruit bats as potential carriers of Ebola virus. a, Dates and locations of animal-trapping sites (blue) and of Ebola virus outbreaks among humans (red stars) in Gabon and the Republic of the Congo. b, Phylogeny of Ebola viruses inferred from RNA polymerase sequences. Values below branches are bootstrap posterior probabilities (left of slash; values less than 0.5 not shown); bootstrap percentages were obtained by maximum parsimony (right of slash; values under 50% not shown). (GenBank accession numbers: DQ205109–205115.) Sequences of the subtype Zaire (red) share five nucleotide signatures in positions 1,755 (T), 1,800 (G), 1,830 (T), 2,002 (A) and 2,003 (C) of the complete coding sequence of the gene encoding RNA polymerase. c, Geographic distribution (inside coloured lines) of the fruit bats *Hypsignathus monstrosus* (blue), *Eidolon helvum* (red) and *Myotis torquatus* (yellow).



### Isolation of Hendra virus from pteropid bats: a natural reservoir of Hendra virus

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Bats: Important Reservoir Hosts of Emerging Viruses

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**INTRODUCTION**

The remarkable mammals known as “bats” and “flying foxes” (order Chiroptera [“hand wing”]) may be the most abundant, diverse, and geographically dispersed vertebrates (Table 1). Although a great deal is known about them, detailed information is needed to explain the astonishing variations of their anatomy, their lifestyles, their roles in ecosystems ecology, and their importance as reservoir hosts of viruses of proven or potential significance for human and veterinary health.

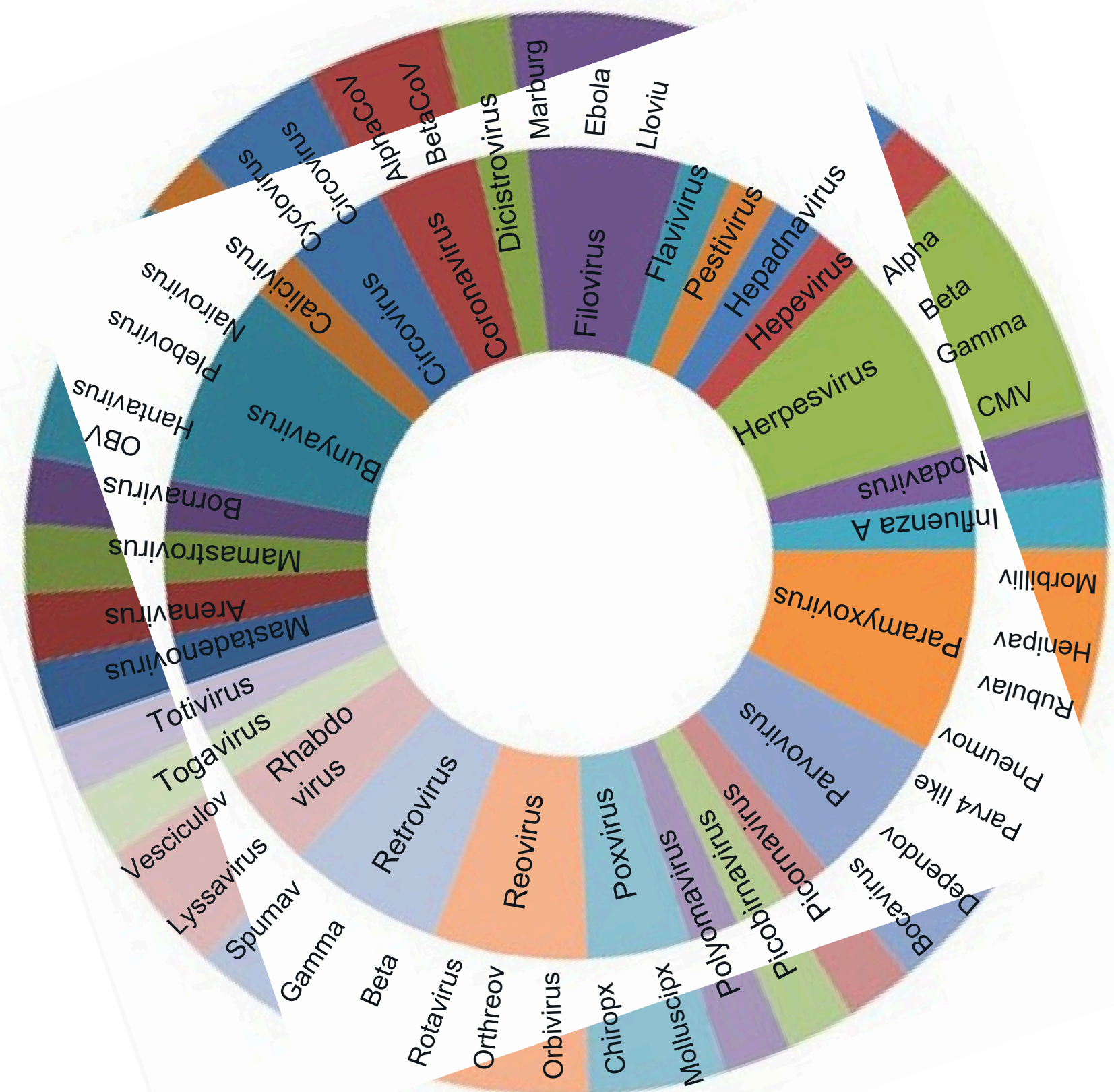
Bats fly with wings which range in span from 130 mm to 2 m. Bats of various species feed on insects, mammals, fish, blood, fruit, and pollen. Bats of most species echolocate to navigate and to find prey. Bats are found on all continents except Antarctica. Bats also are being increasingly recognized as reservoir hosts for viruses which can cross species barriers (i.e., “spill

over”) to infect humans and other domestic and wild mammals. Nonetheless, studies of the natural histories of bats and their importance as reservoir hosts of zoonotic viruses largely have been underappreciated and underfunded, except for their role in maintaining and transmitting rabies virus. Irrespective of the negative public perception of bats, they are critical elements of all terrestrial biotic communities. They help control insects, reseed cut forests, and pollinate plants that provide food for humans and other species, and their guano is used as a fertilizer and for manufacturing soaps, gasoline, and antibiotics (21, 69, 83). Bat echolocation and signal processing have provided models for sonar systems (112, 130).

Myths and misunderstandings about the roles of bats in ecosystems and their danger to other species as hosts of rabies virus have led to efforts to extirpate bat populations, with serious consequent effects on insect control and crop production, without coincidental reduction in the already low incidence of rabies virus transmission by bats (93).

This paper summarizes what is known about viruses isolated from bats. Although there is serologic evidence for infection of bats with many viruses (see, for example, references 82 and 101), we will focus here only on the 66 viruses that have been

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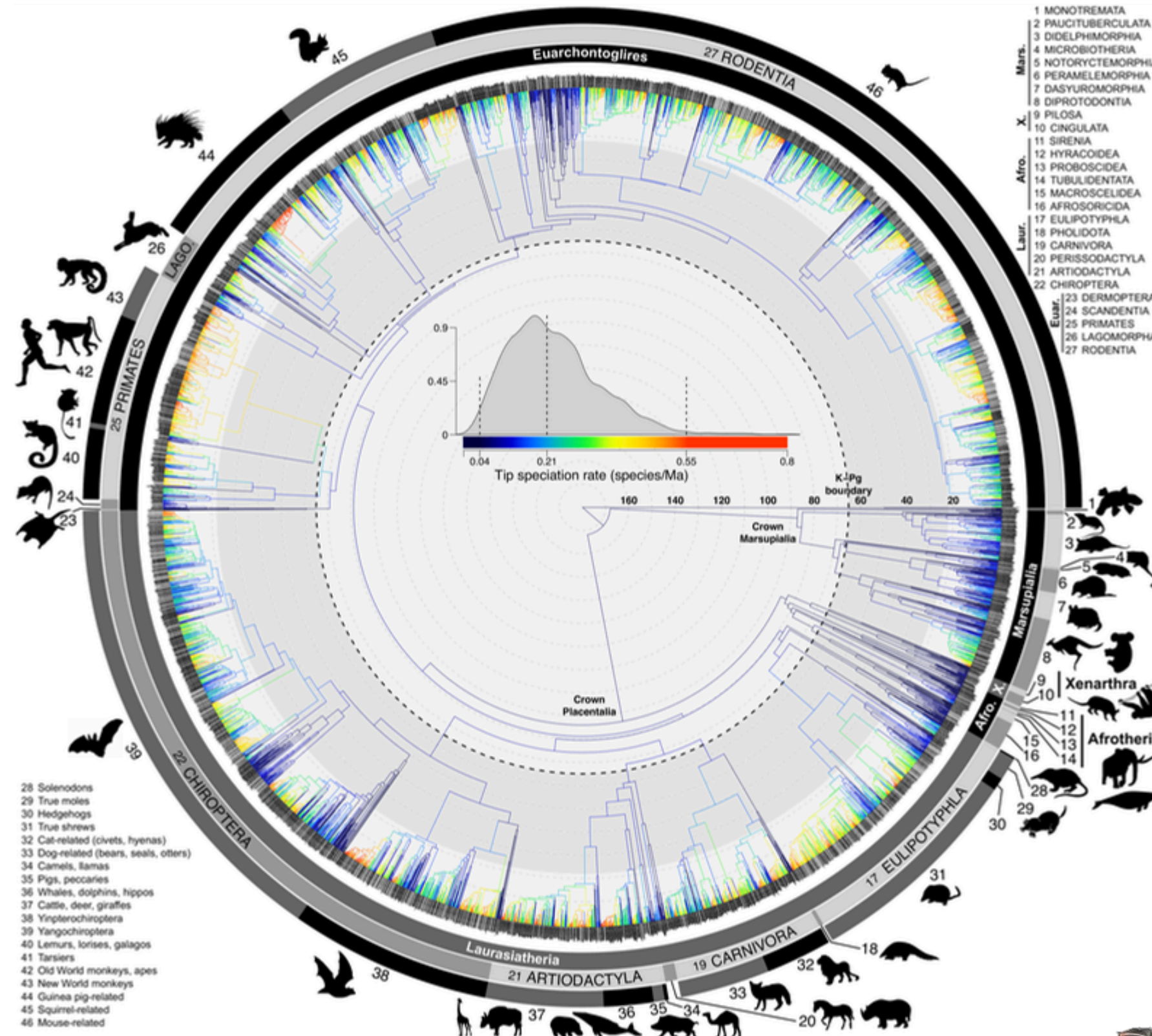


A large crowd of young people at a music festival, many with their hands raised in the air, looking towards a stage in the background.



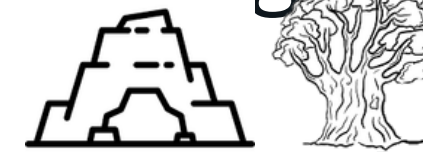


# Bats and their virome



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- More than 1400 species in 18 families
- In Europe 55-57 species in 5 families
- Diverse environments
- Diverse ecological niches
- Diverse feeding habits

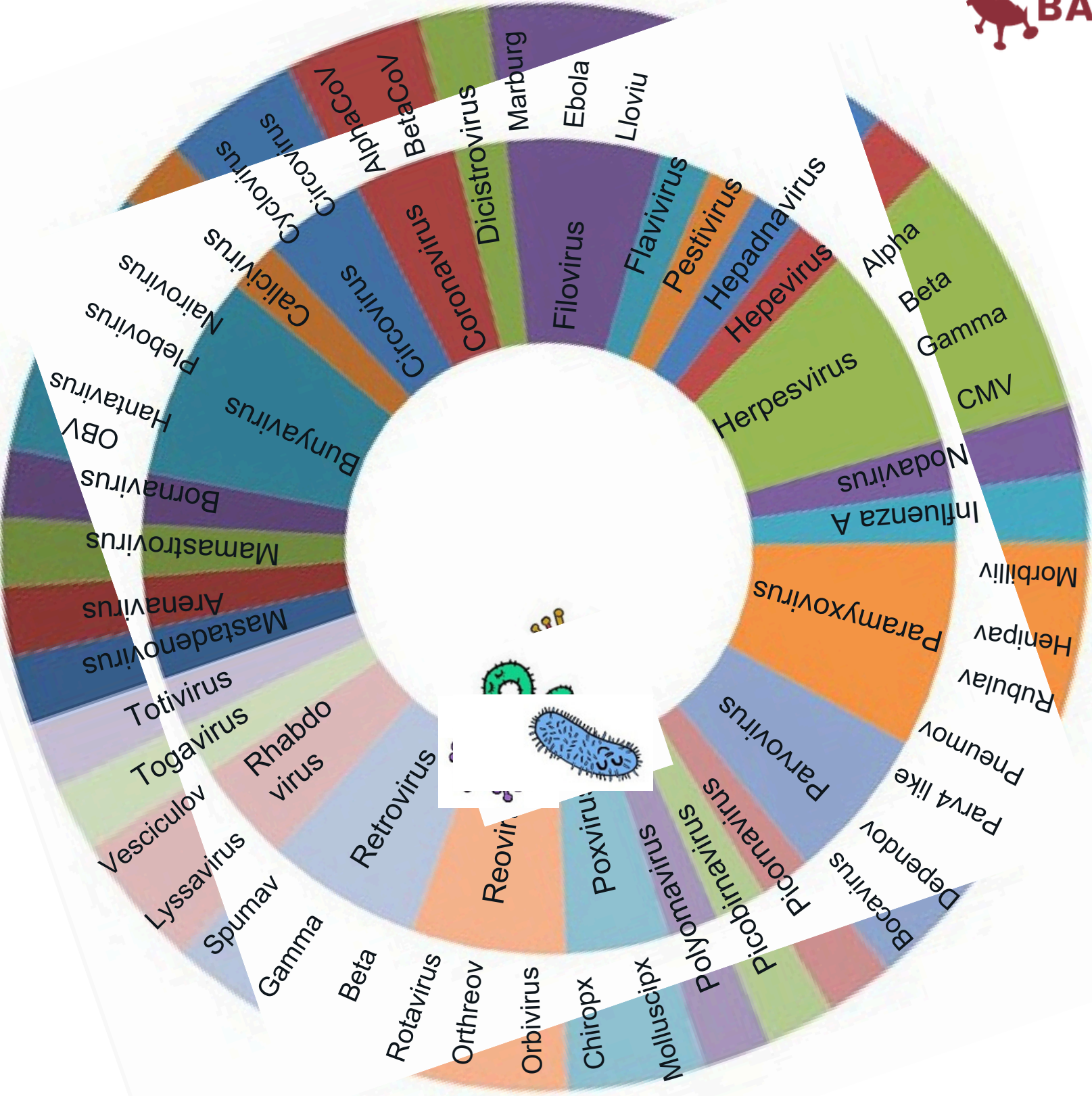
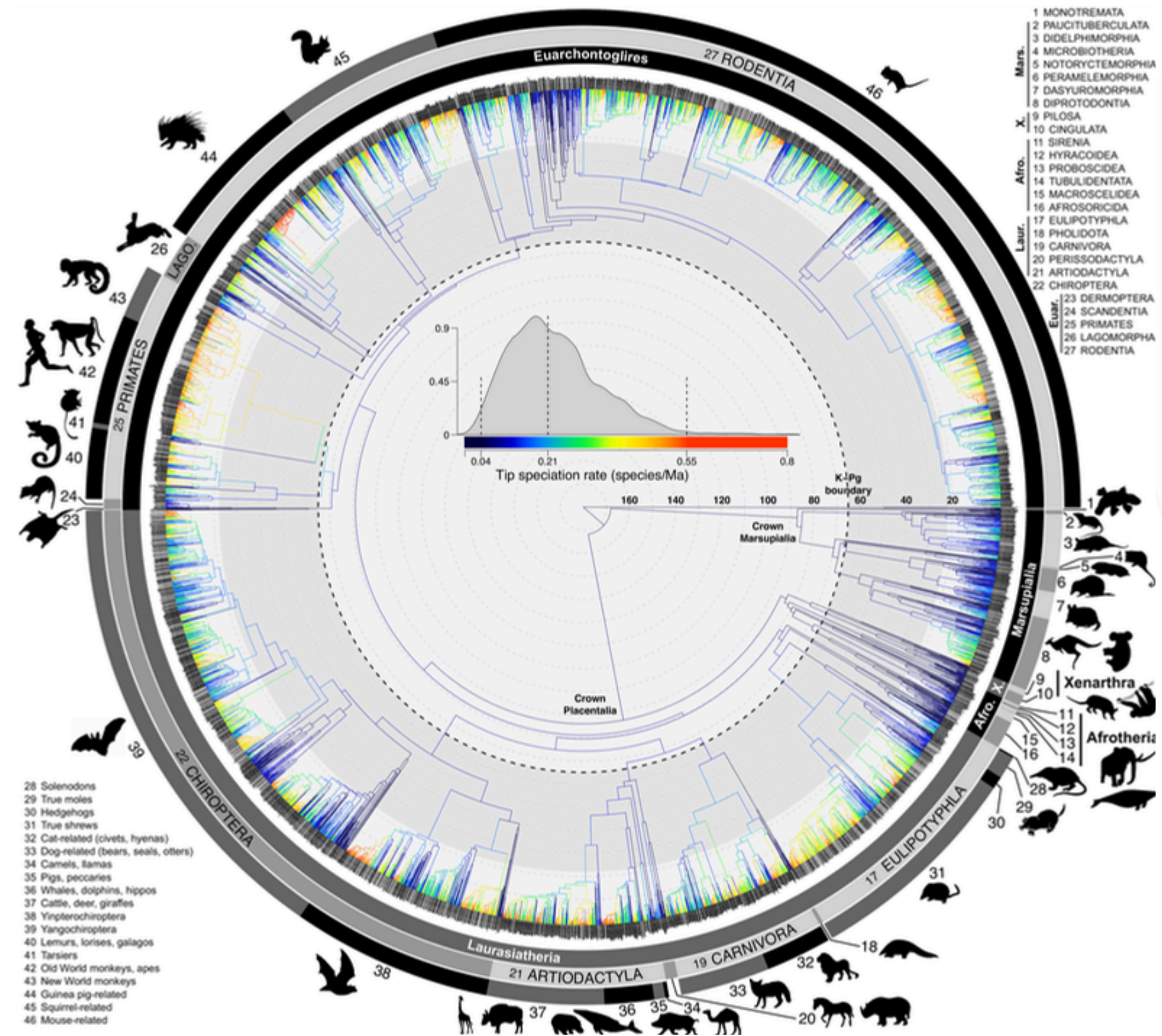


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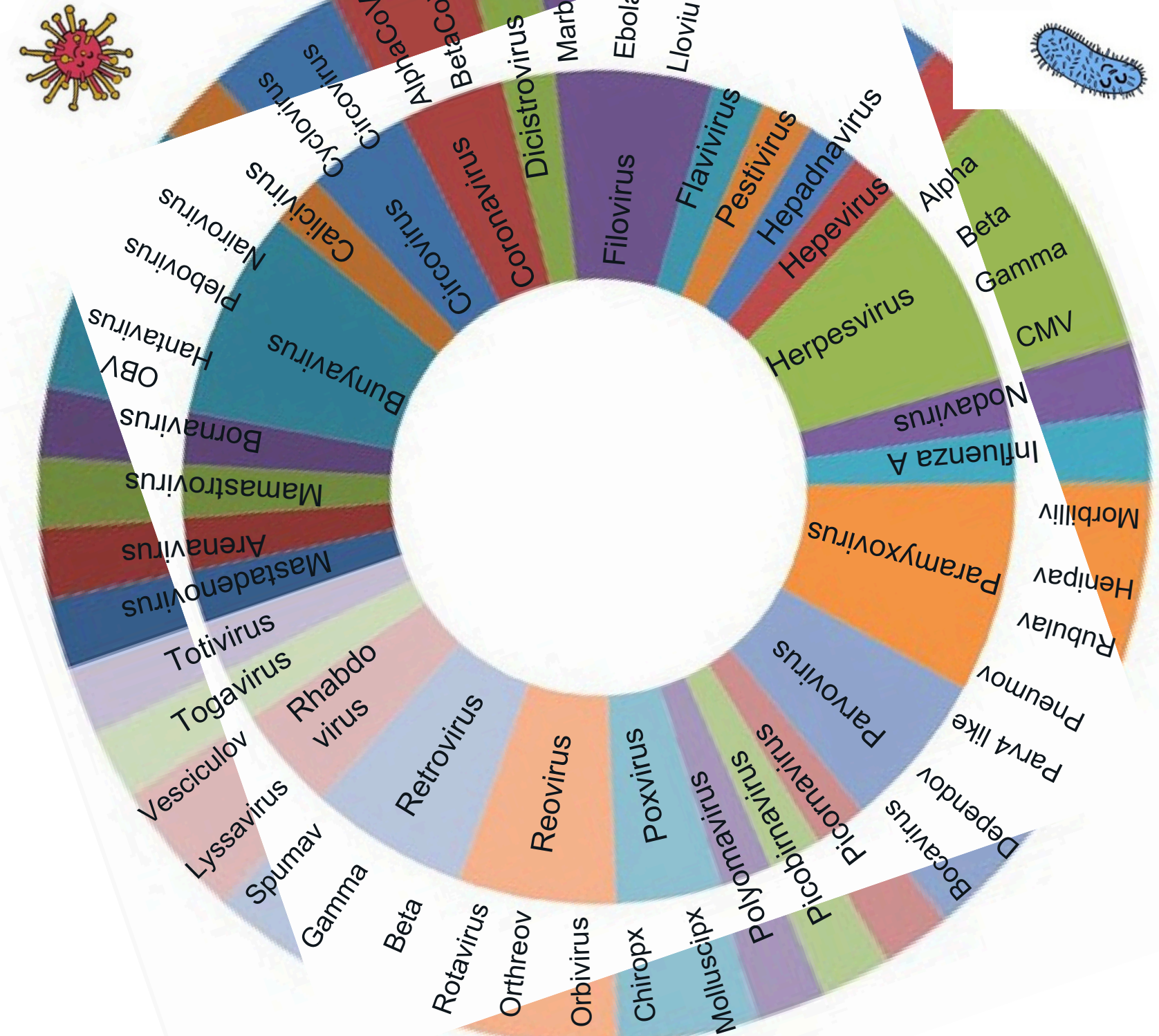
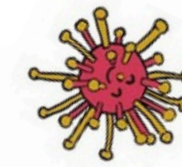
[doi.org/10.1371/journal.pbio.300049](https://doi.org/10.1371/journal.pbio.300049)



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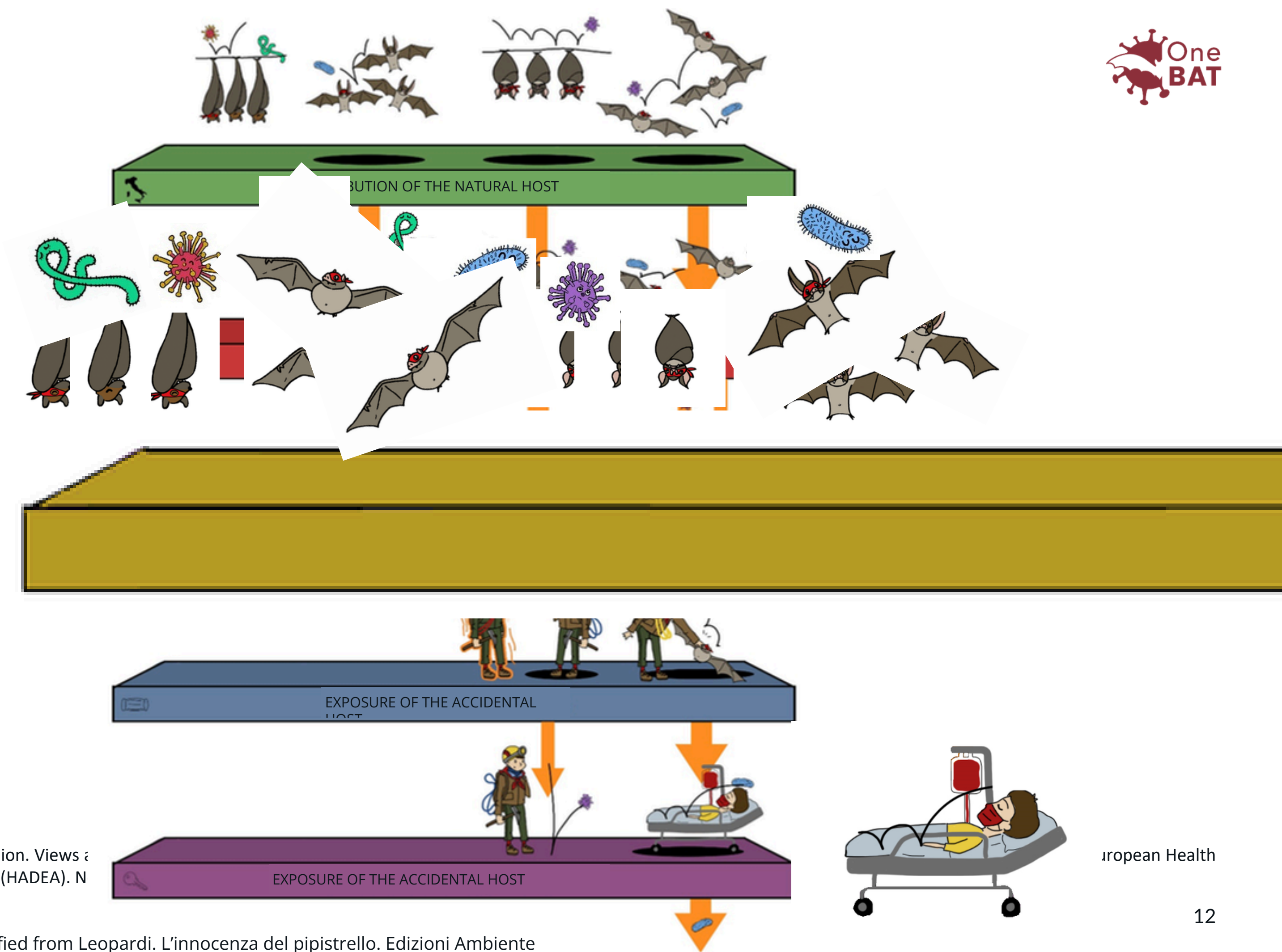
of the European Union or the European Health





Use of the European Union or the European Health



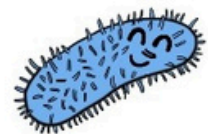




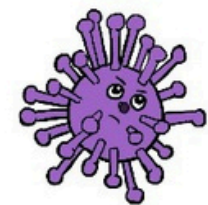
In Europe there are 55-57 species divided in 5 families



## DISTRIBUTION OF THE NATURAL HOST



LYSSAVIRUS



CORONAVIRUS



FILOVIRUS



HENIPAVIRUS



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In Europe there are 55-57 species divided in 5 families



DISTRIBUTION OF THE NATURAL HOST

Henipavirus

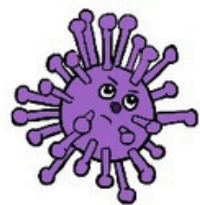
Filovirus

Lyssavirus

Coronavirus



LYSSAVIRUS



CORONAVIRUS



FILOVIRUS



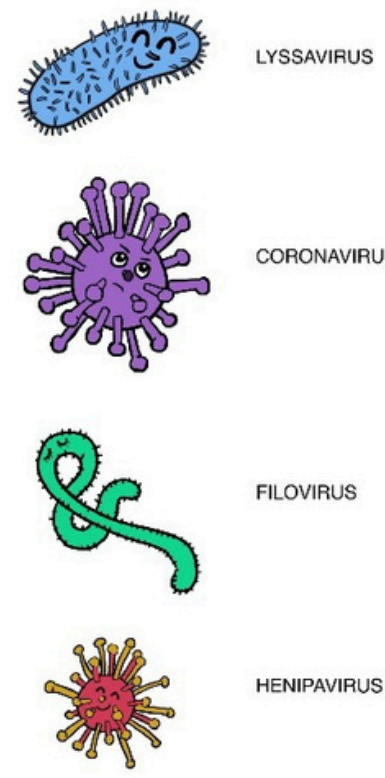
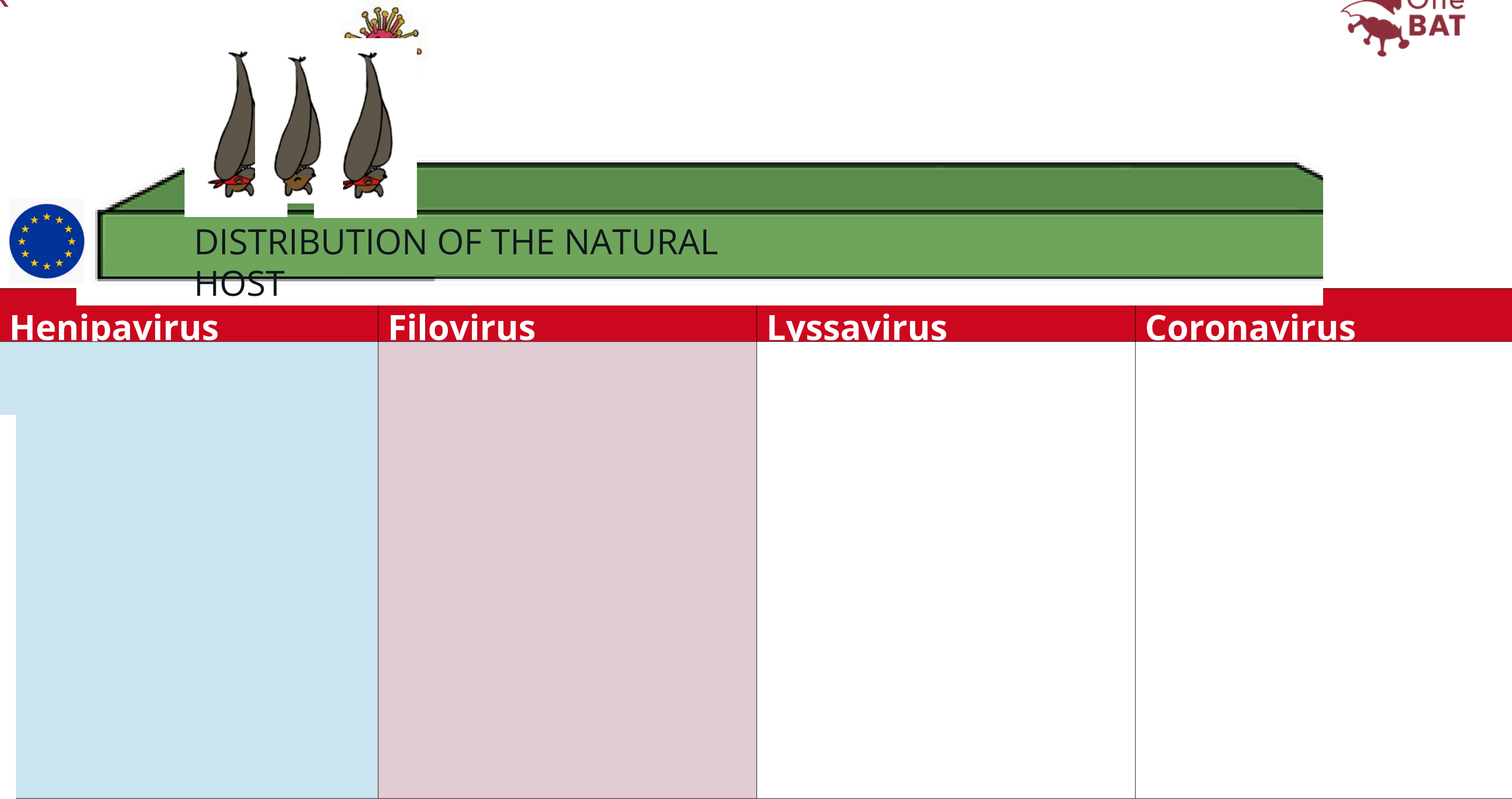
HENIPAVIRUS



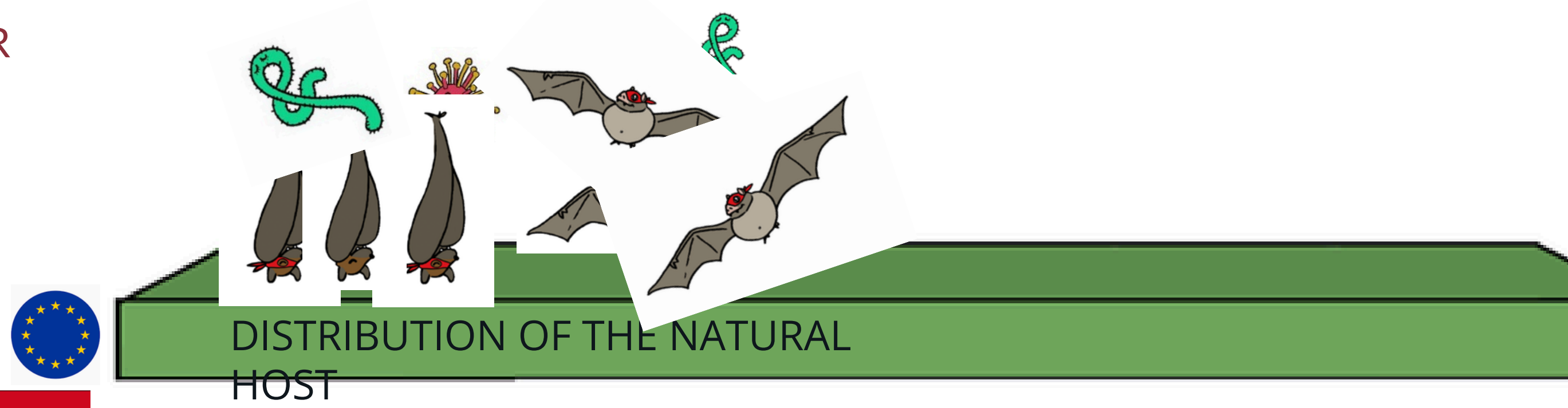
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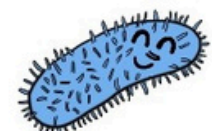




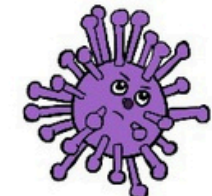




| Henipavirus | Filovirus                                                                                     | Lyssavirus | Coronavirus |
|-------------|-----------------------------------------------------------------------------------------------|------------|-------------|
|             | <b>LLOV</b><br><i>Miniopterus schreibersii</i><br><b>MARV</b><br><i>Rousettus aegyptiacus</i> |            |             |



LYSSAVIRUS



CORONAVIRUS

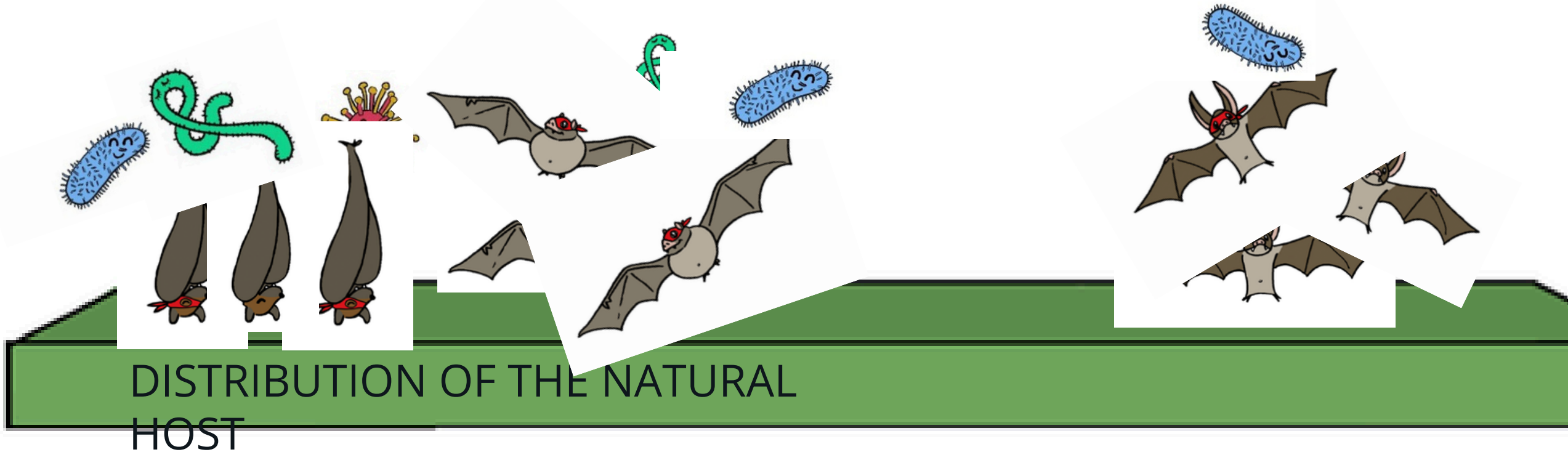


FILOVIRUS

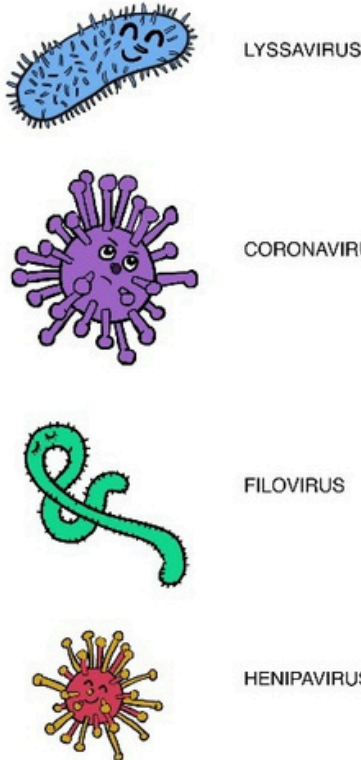


HENIPAVIRUS

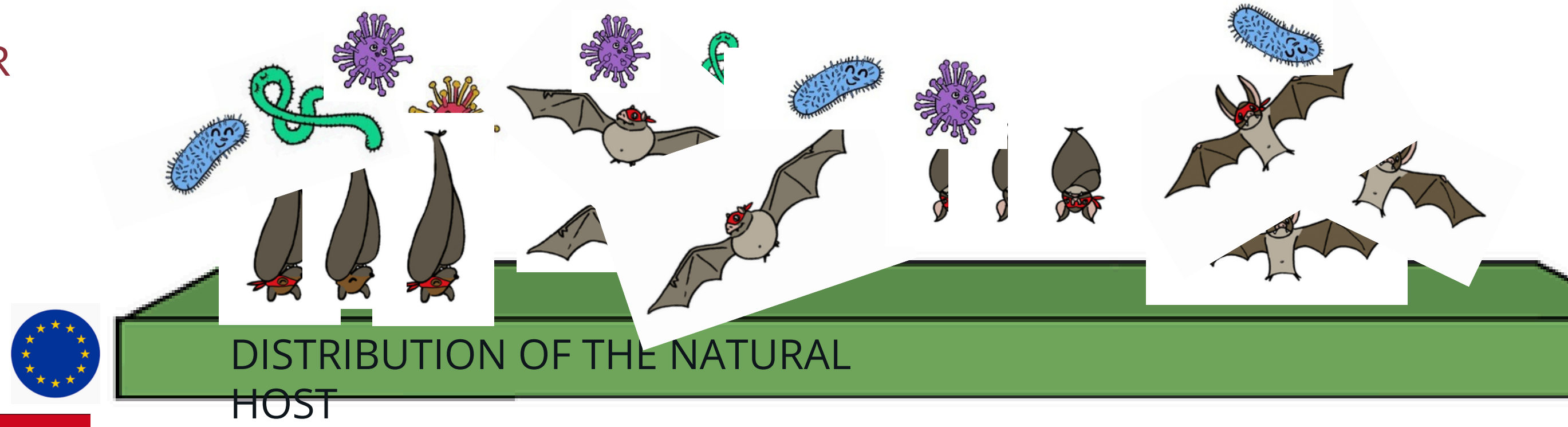


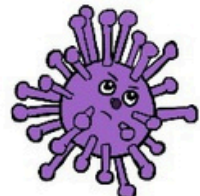


| Henipavirus | Filovirus                                                                                     | Lyssavirus                                                                                                                                                                                                                                  | Coronavirus |
|-------------|-----------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------|
|             | <b>LLOV</b><br><i>Miniopterus schreibersii</i><br><b>MARV</b><br><i>Rousettus aegyptiacus</i> | <b>EBLV1</b><br><i>Eptesicus serotinus</i><br><b>EBLV2</b><br><i>Myotis daubentonii</i><br><b>BBLV</b> <i>Myotis nattereri</i><br><b>LLEBV</b> <b>WCBV</b><br><i>Miniopterus schreibersii</i><br><b>LBV</b><br><i>Rousettus aegyptiacus</i> |             |
|             |                                                                                               |                                                                                                                                                                                                                                             |             |





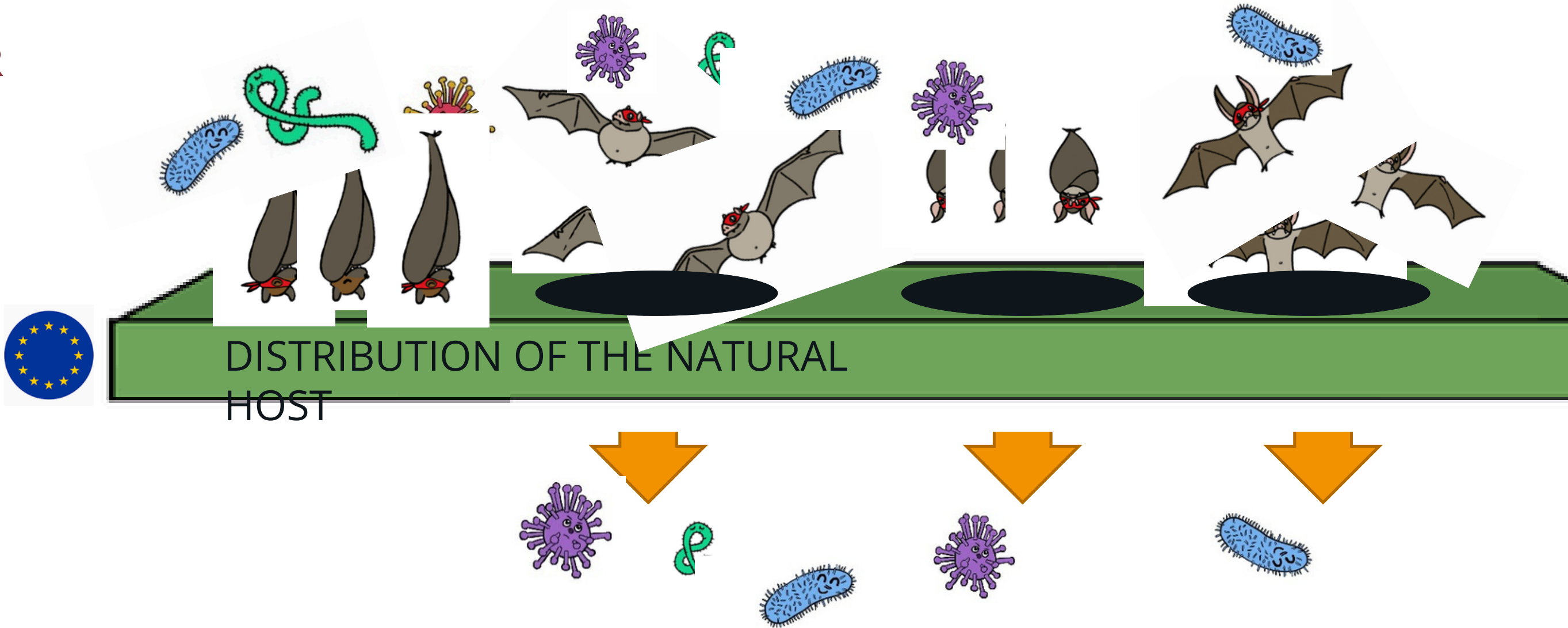


-  LYSSAVIRUS
-  CORONAVIRUS
-  FILOVIRUS
-  HENIPAVIRUS

|  |                                                                                               |                                                                                                                                                                                                                                      |                                                                                                                                                                                                                                                                                                     |
|--|-----------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|  | <b>LLOV</b><br><i>Miniopterus schreibersii</i><br><b>MARV</b><br><i>Rousettus aegyptiacus</i> | <b>EBLV1</b><br><i>Eptesicus serotinus</i><br><b>EBLV2</b><br><i>Myotis daubentonii</i><br><b>BBLV</b> <i>Myotis nattereri</i><br><b>LLEBV WCBV</b><br><i>Miniopterus schreibersii</i><br><b>LBV</b><br><i>Rousettus aegyptiacus</i> | <b>Alphacoronavirus</b><br><i>Myotis spp.</i> ,<br><i>Pipistrellus spp.</i> ,<br><i>Miniopterus schreibersii</i><br><br><b>Betacoronavirus</b><br><i>Rhinolophus spp.</i> ,<br><i>Eptesicus serotinus</i> ,<br><i>Pipistrellus spp.</i> ,<br><i>Hypsugo savii</i> ,<br><i>Rousettus aegyptiacus</i> |
|--|-----------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|



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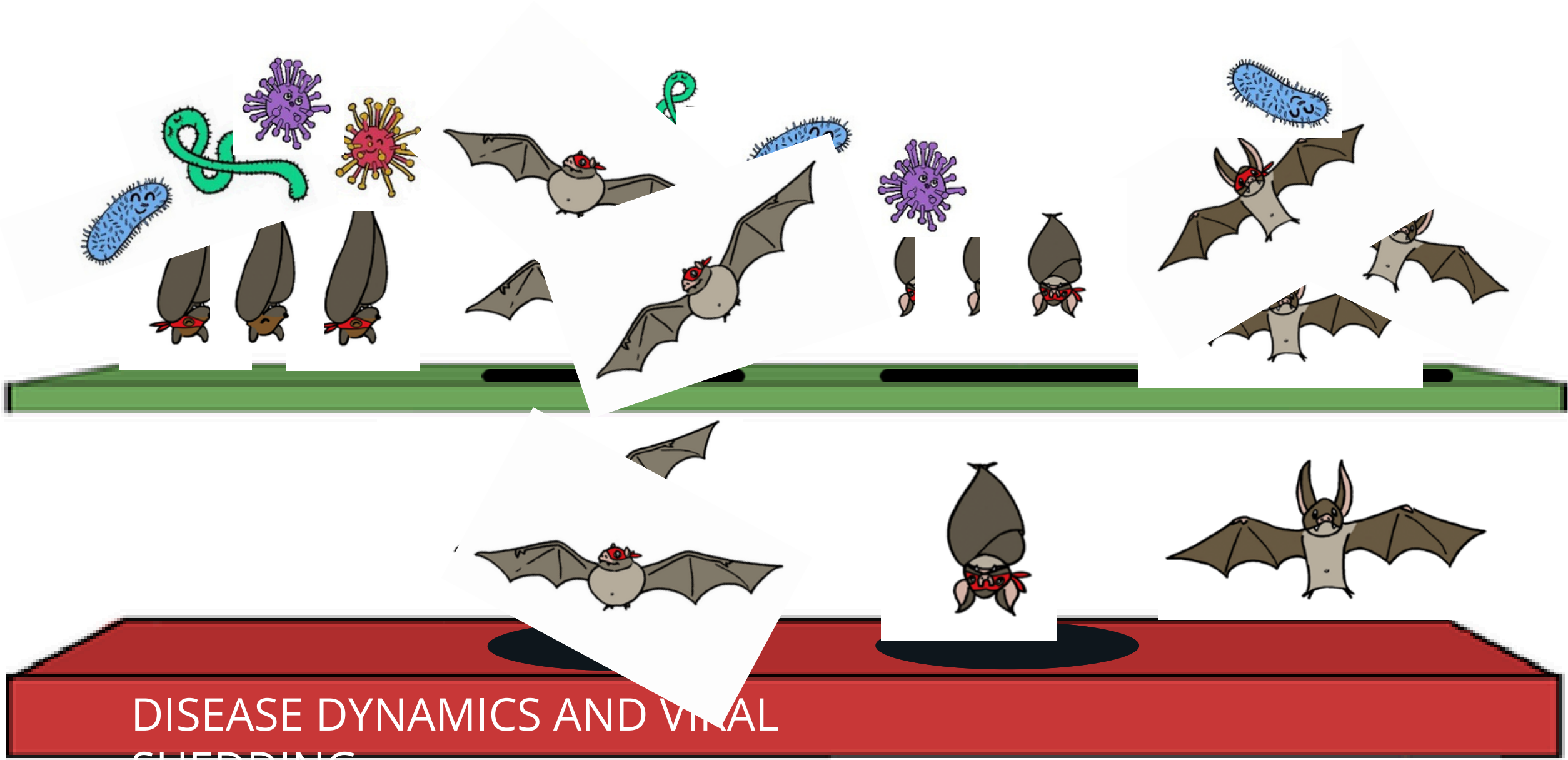


- LYSSAVIRUS
- CORONAVIRUS
- FILOVIRUS
- HENIPAVIRUS

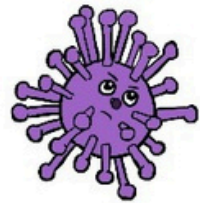


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LYSSAVIRUS



CORONAVIRUS



FILOVIRUS



HENIPAVIRUS



Shedding from  
saliva  
Low prevalence  
Seasonality



Shedding from feces and  
saliva/aerosol  
High prevalence

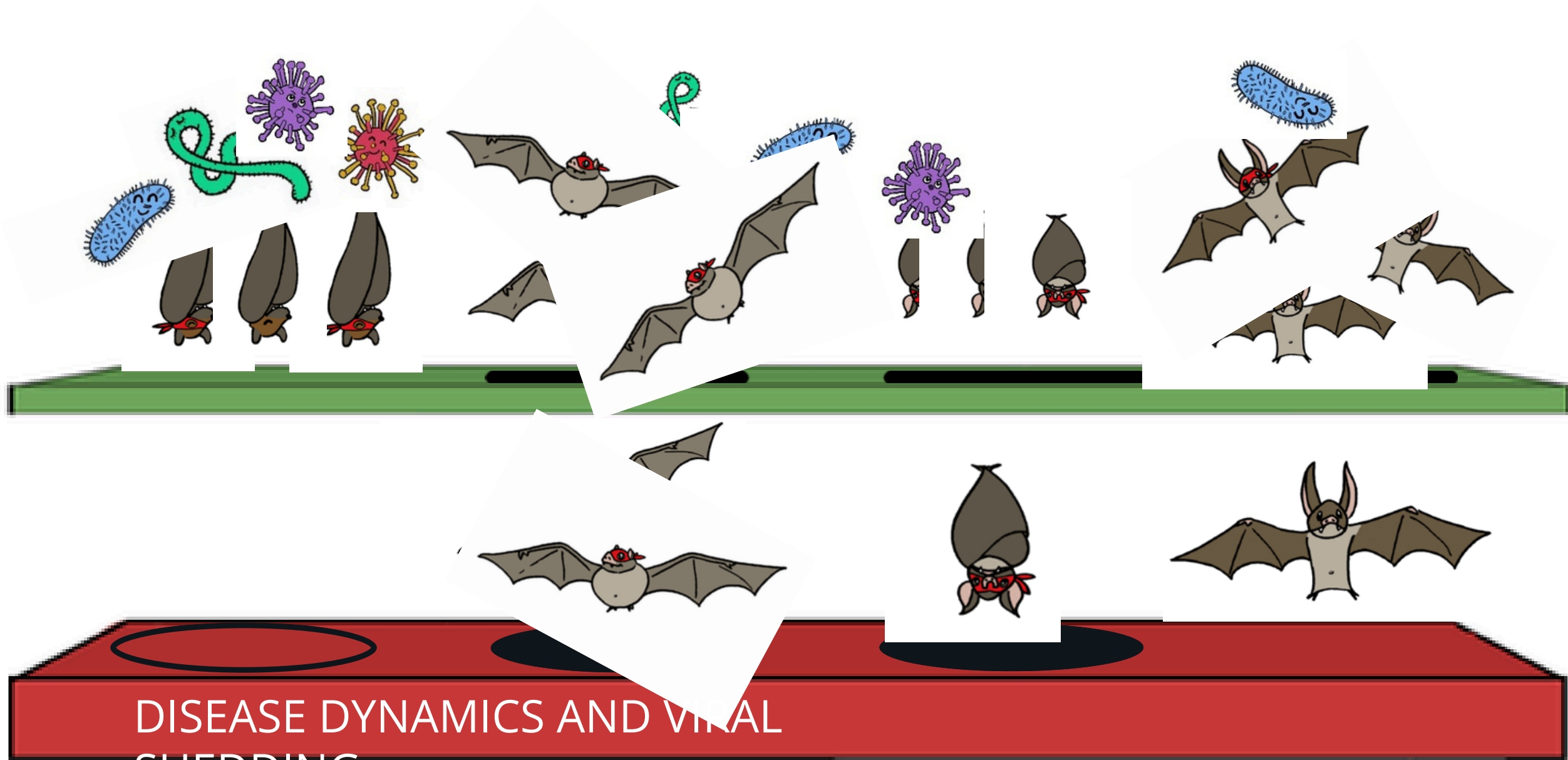


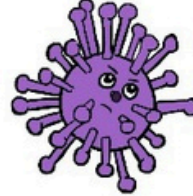
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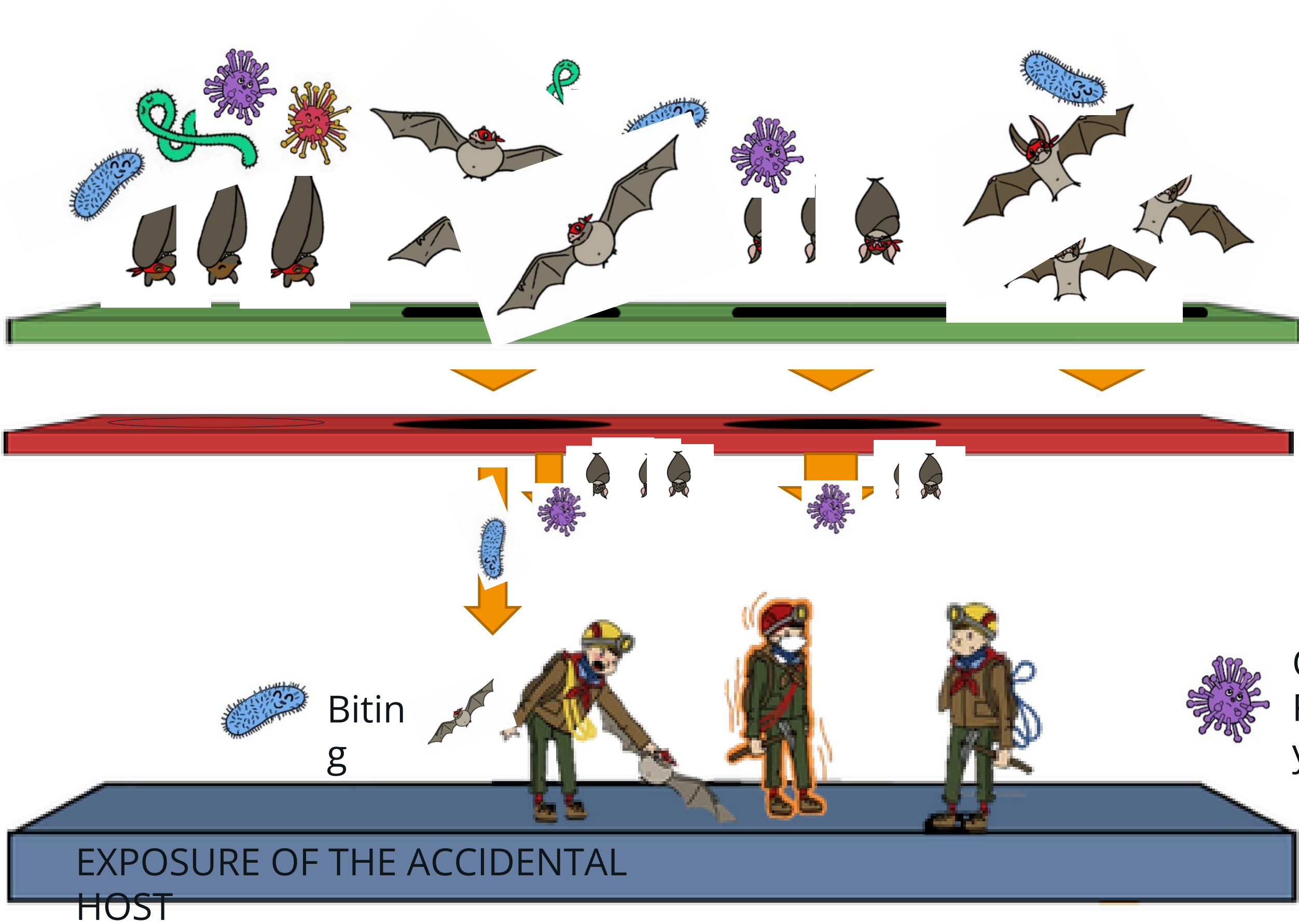
-  LYSSAVIRUS
-  CORONAVIRUS
-  FILOVIRUS
-  HENIPAVIRUS



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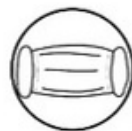
SPILLOVER



- LYSSAVIRUS
- CORONAVIRUS
- FILOVIRUS
- HENIPAVIRUS

Bitin  
g

Oro-fecal  
Respirator  
y

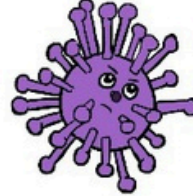


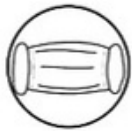
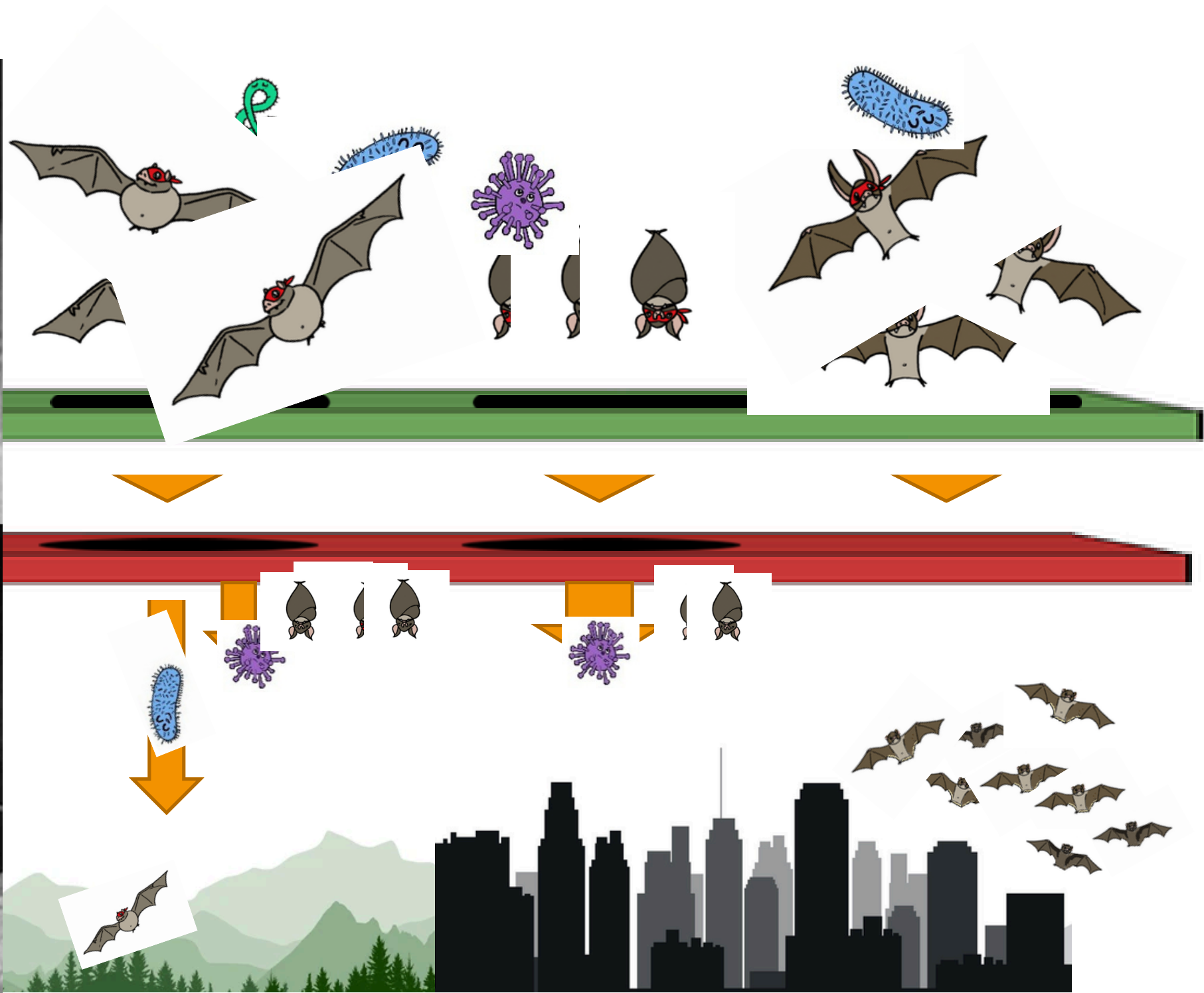
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European Health

SPILLOVER



-  LYSSAVIRUS
-  CORONAVIRUS
-  FILOVIRUS
-  HENIPAVIRUS



EXPOSURE OF THE ACCIDENTAL  
HOST

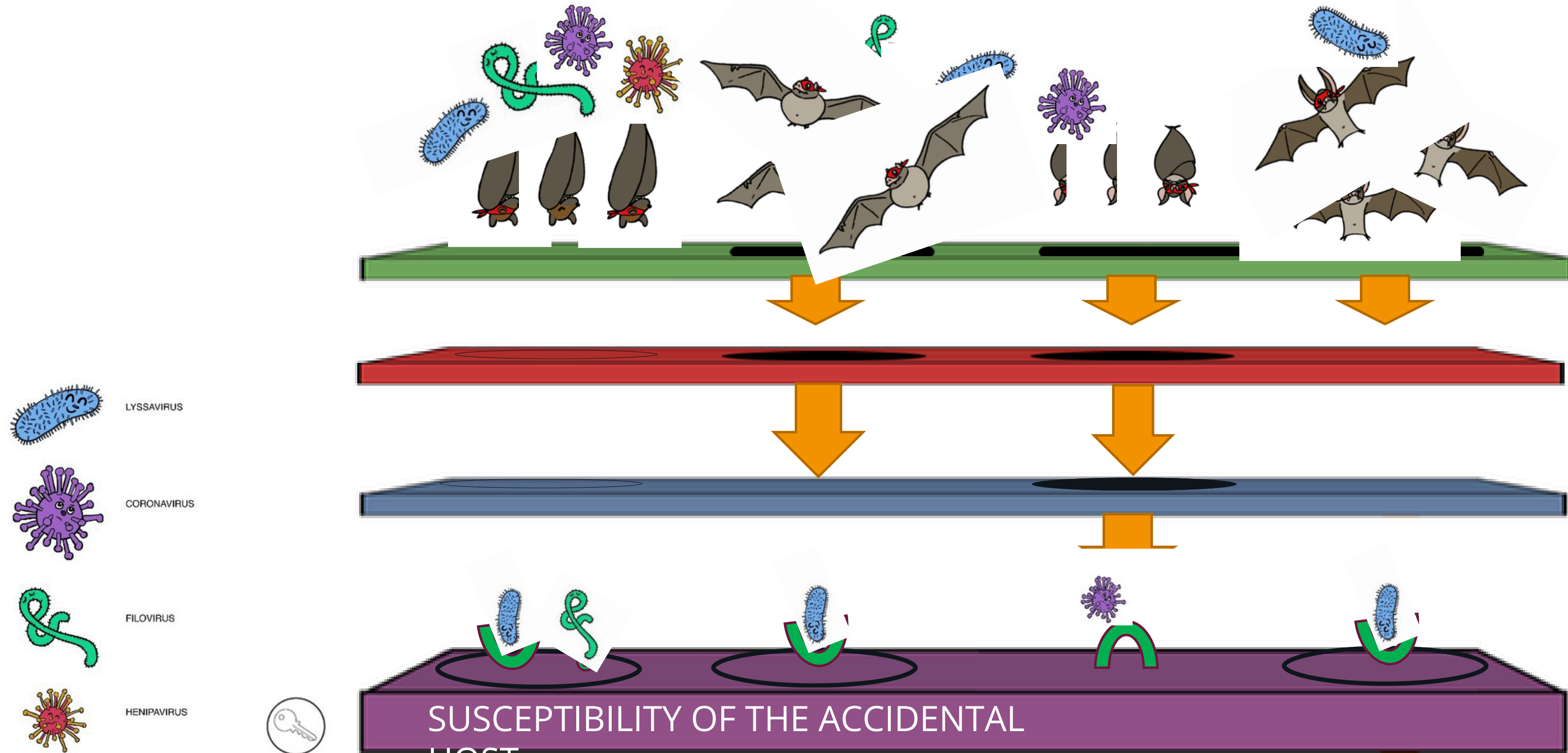


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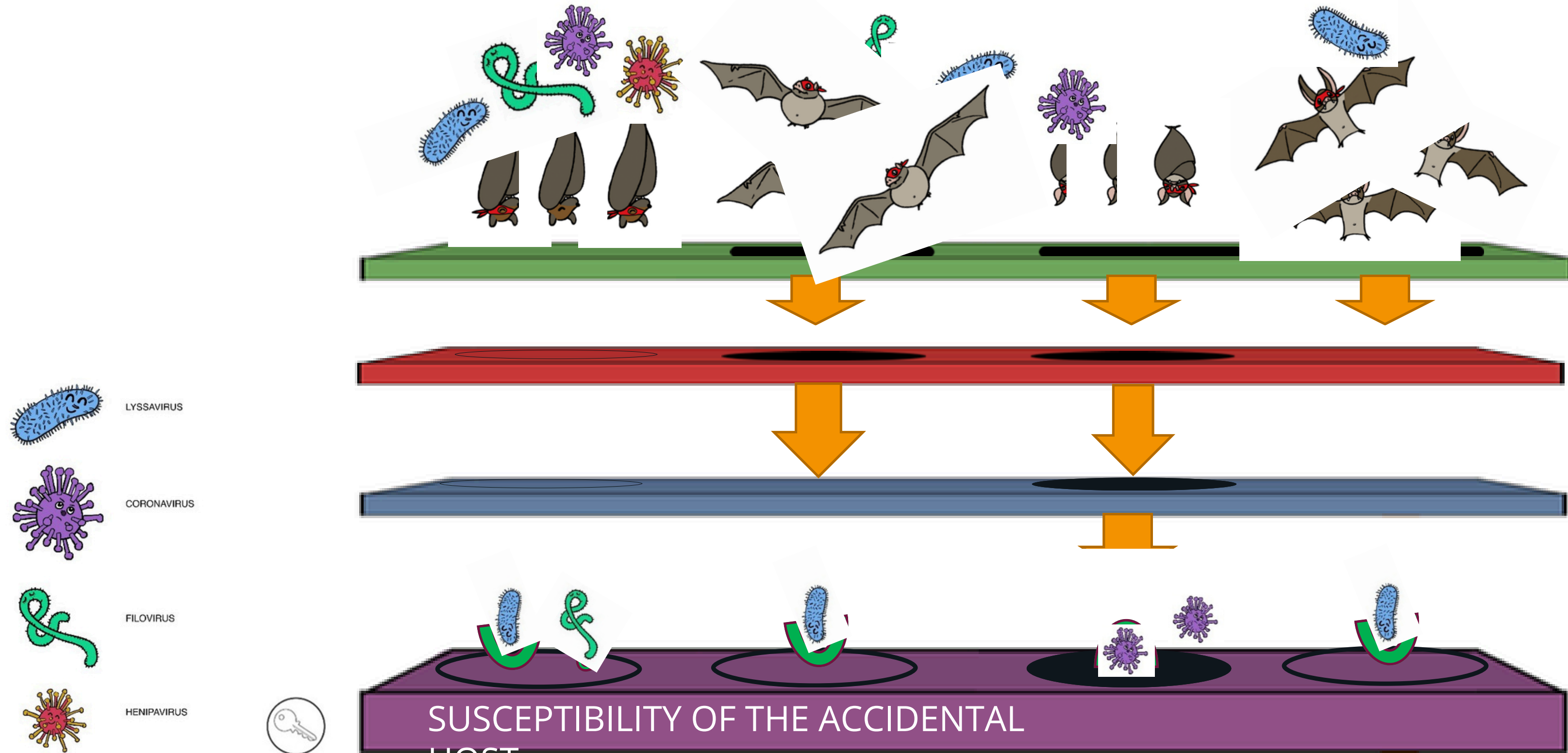
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# SPILLOVER

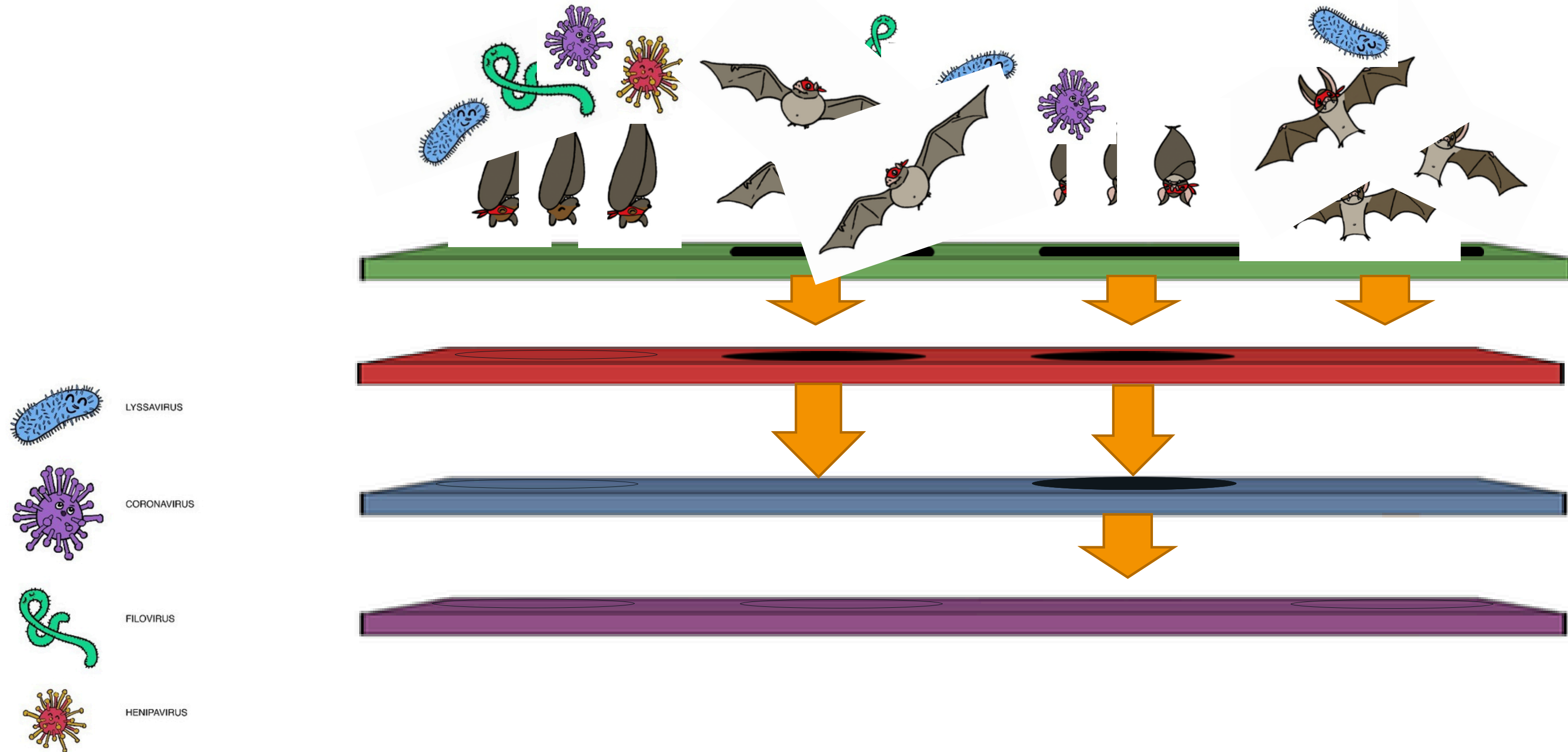


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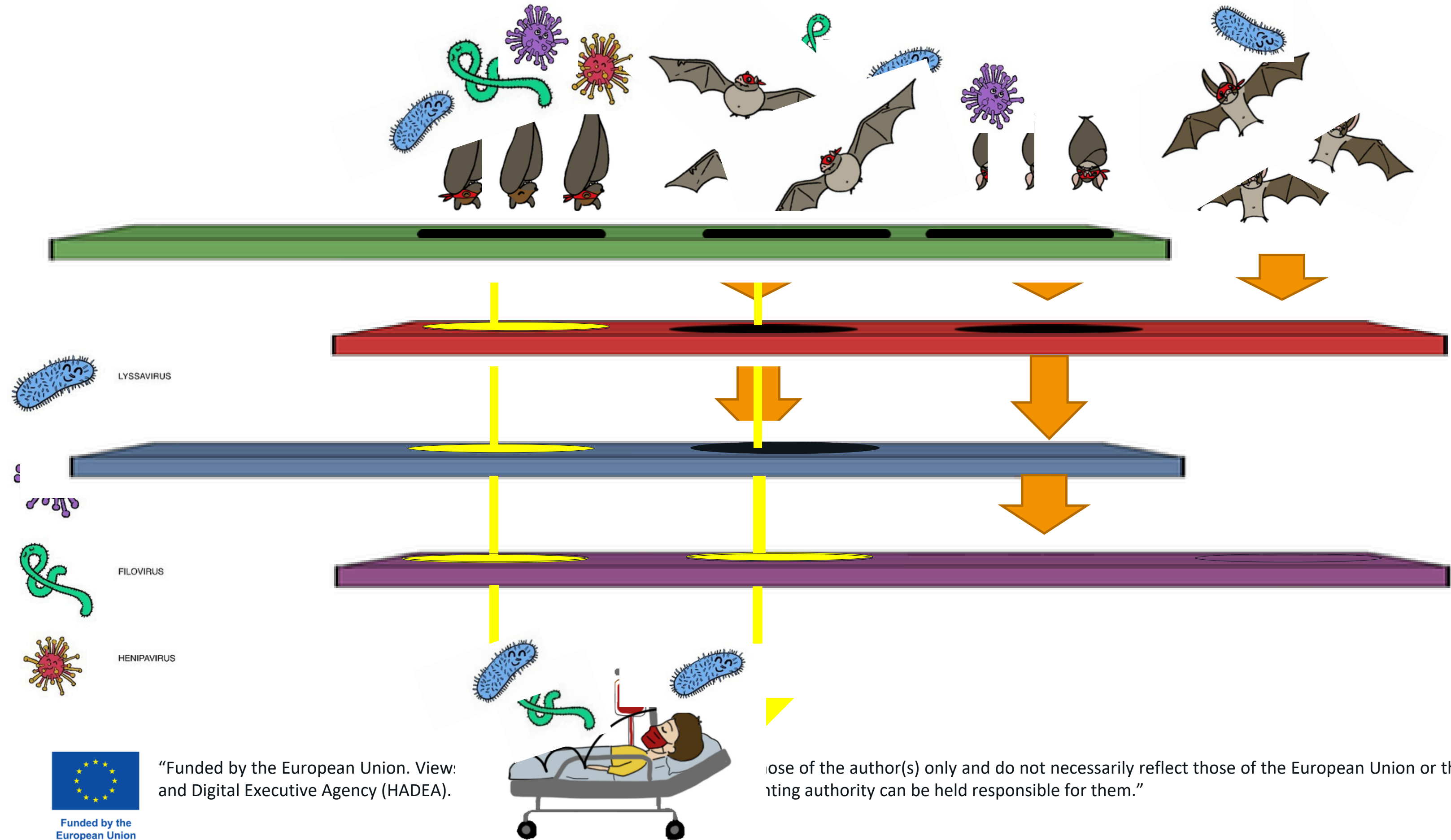
# SPILLOVER



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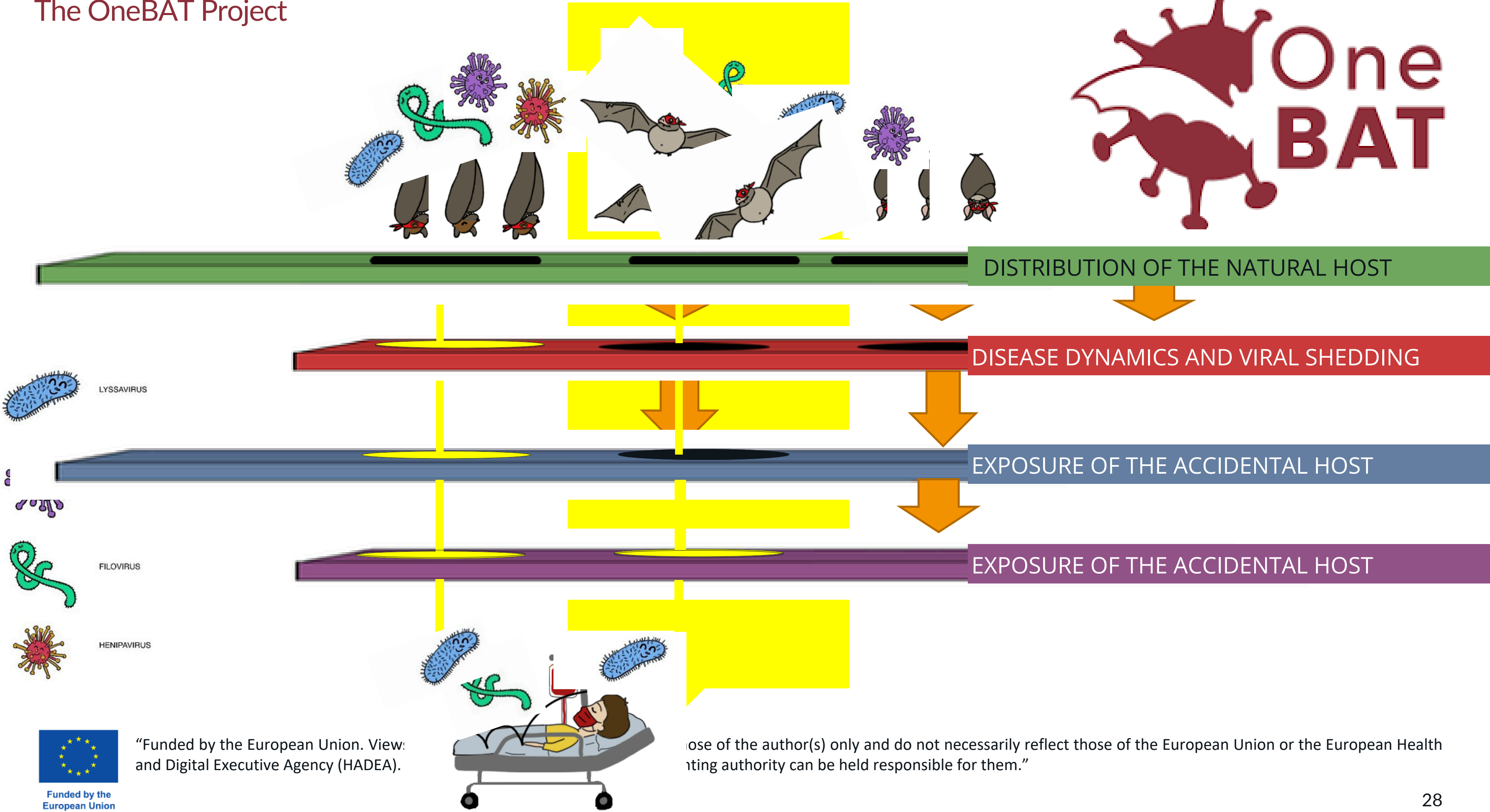
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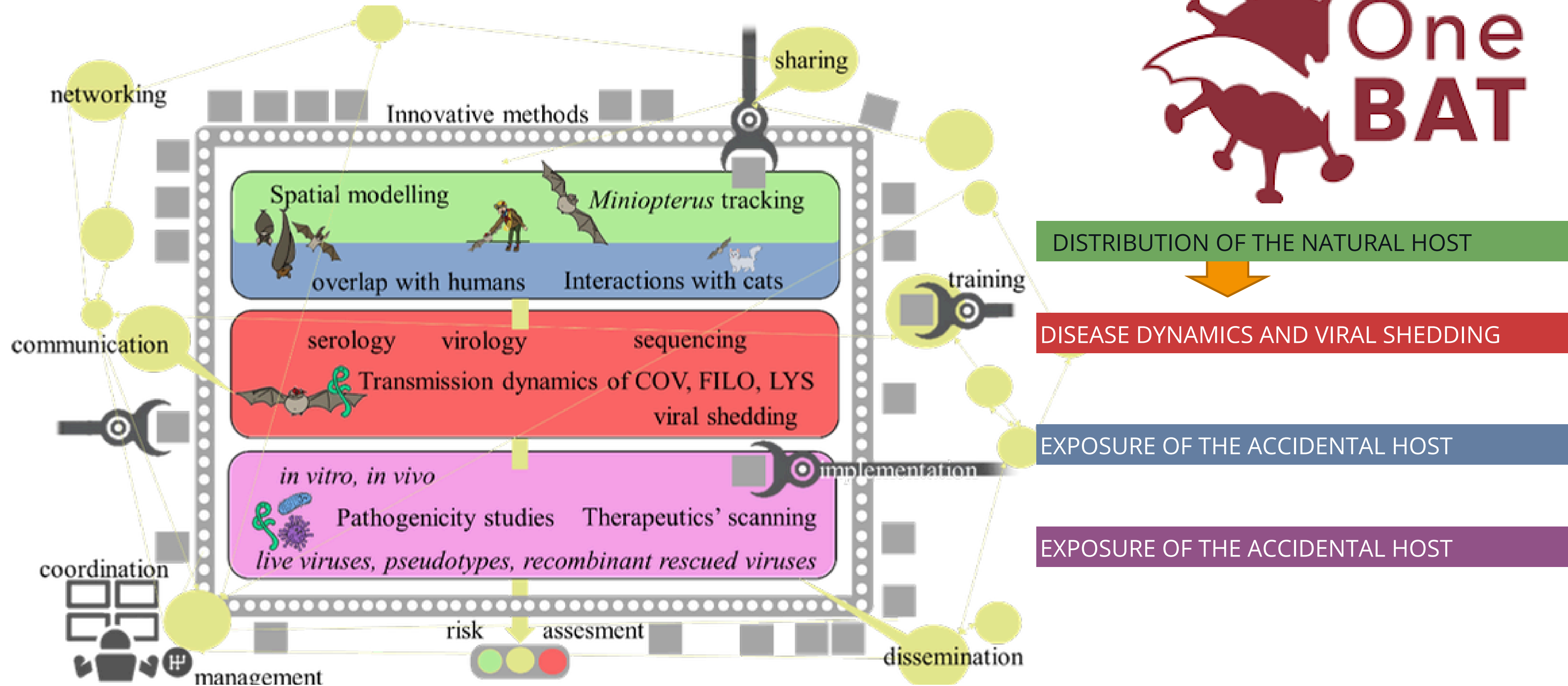




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[sleopardi@izsvenezie.it](mailto:sleopardi@izsvenezie.it)

*Thanks for your attention!*



[www.onebat.eu](http://www.onebat.eu)



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