Project closing report

Renewing the virus taxonomy in the age of metagenomics

(Balázs Harrach)

Our research started with grant NN128309 in 2018. 09. 01. but it got a new number (NN140356) when the Veterinary Medical Research Institute became independent from the Centre for Agricultural Research in 2021. 04. 01. Thus, our first publications refer to that original number.

New molecular techniques, especially metagenomics, have revealed a wealth of new viral sequences. The International Committee on Taxonomy of Viruses (ICTV) has decided that these can be a basis for the establishment of official virus species (Simmonds et al, 2017). Thus, our aim was to gain new sequences with different methods and renew the virus taxonomy of DNA viruses by considering all available viral sequences. Our main goal was to characterize the diversity of selected DNA viruses of animals as much as possible to understand better their evolution and to help the establishing of a renewed, official taxonomy for them reflecting their evolutionary past.

PCR screening of a waste array of samples from domesticated animals and wildlife yielded many novel adeno-, cyclo-, circo-, polyoma-, herpes-, irido-, parvo-, rana- and papillomaviruses (Tarján et al., 2019; de Oliveira et al., 2020; Zadravec et al., 2022; Harrach et al., 2023; Herczeg et al., 2023). Regarding farm animals, we have identified porcine, bovine and fowl adenovirus types never found earlier in Europe, which originated from dead swine, cattle and chicken from field cases thus widening the repertoire of the viruses that must be searched for in ill or dead domesticated animals during routine diagnostic work. We recovered novel emerging and reemerging bovine adenoviruses from Western Hungarian field cases (diarrhoea, enteric haemorrhages, sudden death of young calves) (Vidovszky et al., 2022), As bat viruses tend to infect man and domesticated animals, we studied the viruses harboured by bats. In a large comparative work, we achieved the detection and genetical characterization of circoviruses from more than 80 bat species from four continents (Vidovszky et al., 2023). CRESS (circular Rep-encoding single stranded) DNA viruses seem to have an almost "unlimited" number of hosts (even plants and primitive eukaryotes beside invertebrate and vertebrate animals). We ourselves amplified many CRESS DNA viruses from vertebrates (carnivores, bats, tortoise, frog, penguin, etc.) that turned out not belonging to *Circoviridae*. These viruses may origin from the water or the prey/food of the sampled vertebrates but not propagating in them. We characterized iridoviruses of reptiles and prey insects to understand possible host switches. While most adenoviruses have a restricted host range, psittacine adenoviruses detected in parrots kept in captivity in Slovenia and the USA proved that most of them are capable to cross the species barrier (Zadravec et al., 2022). Amplifying viral genome fragments from different hosts or by metagenomics, we run often into the problem that the amplified fragment may originate from a viral genome fragment inserted into the chromosome but not from an actively propagating ("living") virus. This happened with alloherpesvirus, CRESS DNA virus, adintovirus, adomavirus genome fragments, thus clearly, certain additional molecular and bioinformatics techniques are needed to be applied to confirm results suggesting the existence of such living viruses. Obviously, the genome-inserted remaining of ancient viruses cannot be

considered as actual pathogens meaning danger for our livestock. However, they can give hints about the evolutionary past of certain virus lineages (paleovirology.)

All these novel alloherpes-, CRESS DNA, polyoma-, testadenovirus, etc. findings in amphibians, reptiles, birds, carnivores, ruminants, etc. proved that even with the newest techniques available, we still just see the surface of the virus diversity present in vertebrates. The novel molecular techniques seem to prove that certain viruses occur only in vertebrates (like adenoviruses), but other viruses occur in large number in invertebrates, too (e.g., CRESS DNA viruses) making challenging the drawing of conclusion on the real hosts they propagate in when they are recovered from vertebrate samples.

Overall, our consensus sequence-based PCR systems proved to be very effective for the general detection of novel members of all the respective virus families, even for samples gained by non-invasive ways (faeces samples) (Harrach et al., 2023).

We analysed the **full genome** of a newly found ovine adenovirus (isolated from dead sheep in Hungary) (Vidovszky et al., 2019), the full genome of the single known fish adenovirus (isolated from white sturgeon in the USA) (Doszpoly et al., 2019), and a novel fowl adenovirus type isolated from diseased chicken in Eastern Hungary (Kaján et al., 2019). We also analysed the full genome of an adenovirus found in a polar bear that died in the zoo of Budapest (Böszörményi et al., 2020). We identified and sequenced the first bat polyomaviruses found in European bats (Vidovszky et al., 2020; Surján et al, 2023). The first two horseshoe bat polyomavirus genomes revealed an evolutionary history of intrahost divergence with horseshoe bats distributed across the African and Eurasian continents, i.e. despite the significant geographical distances between the corresponding sampling locations, Hungarian polyomaviruses exhibited high genetic relatedness with previously described Zambian and Chinese horseshoe bat polyomaviruses (Vidovszky et al., 2020). We analysed the full genome of an atadenovirus detected in bearded dragon (Pénzes et al., 2020). It harbours three novel genes encoding proteins of the C-type lectin-like domain superfamily. We gained full genomes of different avian adenoviruses from wild birds (representing all three genera where avian adenoviruses may belong to) (Kraberger et al., 2022; Surplis et al., 2022). Following the genome analysis of Old World monkey adenovirus serotypes simian adenovirus 2 and 17, we published a comparative study on the characterization of simian adenoviruses with three fiber genes and the possible evolution of these fiber genes (Podgorski et al., 2023). These adenoviruses may be well applied as human and veterinary vectors because they can use three different cellular receptor types, thus provide multiple possibilities for artificial fiber swapping and desired cell targeting.

A longer genome sequence was gained from the red-eared slider adenovirus 1, which made possible the acceptance of the sixth genus in *Adenoviridae*: *Testadenovirus* (Harrach et al., 2019) We succeeded with the identification and partial genetic characterisation of a novel alloherpesvirus detected by PCR in a farmed wels catfish (*Silurus glanis*) presenting skin lesions (Tarján et al., 2022).

We published a **phylogenetic analysis** of all available monkeypox virus strands, which shows the close relatedness of contemporary ones from the present epidemic (Benkő et al., 2023b).

Metagenomics yields a quickly growing number of novel virus sequences recently. Earlier, in the ICTV, we decided to accept such virus sequence as valid subjects for classification (under

certain rules) (Simmonds et al., 2017). Thus, our work in the present grant constantly also involved those virus sequences where actual virus isolation has not been yet successful and/or we do not know the true host. We also applied metagenomics successfully during our laboratory work. It yielded a whole new siadenovirus genome from a great tit sample in our lab, thus proving that an adequate enhancement of the relative amount of viral DNA in samples could yield full adenoviral genomes (Gellért et al., 2022). Similarly, we analysed a mastadenovirus full genome gained by metagenomics from a sample of a reindeer died in the UK (Dastjerdi et al, 2022).

I continuously update my online database on the graphical representation of available adenovirus sequences (https://sites.google.com/site/adenoseq/). It shows the sequenced parts of the adenoviruses published and provides links to the hosts in Wikipedia. Its international popularity prompted us to write review papers and book chapters on the adenoviruses and their changing taxonomy (Harrach et al., 2019; Harrach and Benkő, 2021; Benkő et al., 2022a).

We **proposed** to establish a new, sixth genus in *Adenoviridae* (*Testadenovirus*) for the adenoviruses of testudines (turtles, tortoises and sliders), with type species *Pond slider testadenovirus A* and it has been accepted by ICTV (Benkő et al., 2022a).

The **taxonomy** of viruses had a radical renewal in the last years. I had the luck to become the Chair of the Animal DNA Viruses and Retroviruses subcommittee of ICTV. It was decided to publish new summary works, so called reports, on all virus families. My task was the editing of the following chapters on animal DNA viruses, retroviruses and their satellites during the years of the present grant: families *Hepadnaviridae*, *Hytrosaviridae*, *Metaviridae*, *Nimaviridae*, *Nudiviridae*, *Parvoviridae*, *Pseudoviridae*, *Redondoviridae*, *Retroviridae*, and genus *Deltavirus* (Magnius et al., 2018; now in *Kolmioviridae*) (full text and resources on-line: https://ictv.global/report/; summaries in Journal of General Virology: https://www.microbiologyresearch.org/content/ictv-virus-taxonomy-profiles). The Report on the *Adenoviridae* has been prepared personally (Benkő et al., 2022a; https://ictv.global/report/adenoviridae) and is already well cited (59 independent citations). Our team members participated also in the preparation of the Reports on *Herpesviridae* (Gatherer et al., 2021).

As the chair of the Animal DNA Viruses and Retroviruses Subcommittee of the International Committee on Taxonomy of Viruses, I had to deal with all the relevant taxonomical proposals, which was not always without conflicts, but the scientists appreciated these works (Walker et al., 2019, >200 independent citations). In 2019, I handled 12 formal taxonomic proposals to establish one new family, 14 subfamilies, numerous new genera and species. In 2020, I handled 19 proposals to establish 1 new realm, 2 classes, 2 orders, 2 families, 44 genera and 209 species. During the years of the grant, several proposed animal DNA virus families had gained official status: *Adintoviridae*, *Redondoviridae*, etc. A rather serious reorganization was necessary for the family *Parvoviridae*. The revised taxonomy is now independent of the canonical approach based on host association, i.e., there were two subfamilies for vertebrate and invertebrate parvoviruses, respectively, while now a third subfamily has been established based on phylogenetics (Pénzes et al., 2020). I participated in the taxonomic update for avian and mammalian anelloviruses (family *Anelloviridae*; recognition of new members and establishment of species demarcation criteria) (Kraberger et al., 2021; Varsani et al., 2021).

A great advance was when ICTV accepted the **higher taxons** for viruses. Thus, the five earlier official taxon levels have been elevated to 15 (Gorbalenya et al., Nature Microbiology, 2020, IF >15, 140 independent citations). Realms were established as highest taxon levels; originally one for all the RNA viruses and three for the DNA viruses. By now, there are already 6 realms, 4 harbours DNA viruses (*Varidnaviria, Duplodnaviria, Monodnaviria, Adnaviria*), one contains RNA viruses (*Riboviria*), and one contains satellite nucleic acids (*Ribozyviria*) (Koonin et al., 2023). These lineages are supposed to originate and evolve independently. The establishing of the higher taxons opened the possibility to organize (almost) all virus families into different hierarchical taxons. E.g., phylum *Cressdnaviricota* was established for the CRESS DNA virus families and the many still unclassified such viruses found constantly in all eukaryotes (Krupovic et al., 2020).

The most radical change (after a lot of heated discussions) was perhaps the introduction of the obligatory **binomial** (two-tag, Linnaean) virus **species names** (Postler et al., 2017**;** Siddell et al., 2020). This means that in the future, only such species names are allowed to be given. By this year, practically, we finished the renaming of all "obsolete" (other format) virus species names. In the case of *Adenoviridae*, we applied Latinized form, furthermore we renamed a genus (*Atadenovirus*) after the famous Hungarian veterinary virologist Adorján Bartha: *Barthadenovirus*, and also proposed 22 new species (Benkő et al., 2023a). In the case of *Circoviridae* and *Herpesvirales*, the free form binomal species naming was selected. This resulted in the renaming of 1 family (*Herpesviridae* to *Orthoherpesviridae*), 4 genera and 124 species in the order *Herpesvirales* (Benkő et al., 2022b). In the family of *Circoviridae*, 101 species have been renamed and 48 new species established (Varsani et al., 2022). These works were made possible by our continuous chairing the Adenoviridae study group of ICTV, and participating in the Herpesvirales and Circoviridae study groups. Our earlier PhD student who works already in the USA, became the chair of two further study groups (Parvoviridae and Bidnaviridae) and also the member of the Executive Committee of ICTV. Thus, we should be be able to continue our work in virus taxonomy to solve the the later arising problems, too.

We presented our work in international conferences in Mexico, Australia, Spain and Croatia. We published our results in 35 papers in international peer-reviewed journals (total IF = 56.077). So far, five of the papers got >100 independent citations).

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