Simian adenoviruses: Molecular and serological survey in monkeys and humans in Thailand

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ABSTRACT

Simian adenoviruses are in the genus Mastadenovirus of the family Adenoviridae. This family is composed of non-enveloped, double-stranded DNA viruses that infect a wide range of animals. Mastadenoviruses infect mammals, including non-human primates and humans. The close genetic relatedness between simian and human adenoviruses, with its associated potential for the cross-species transmission of zoonotic adenoviruses from monkeys to humans and vice versa, poses important health concerns and thus warrants research. In this study, we performed a molecular survey of adenoviruses in monkeys in Thailand. Most of the monkeys tested here were long-tailed macaques, free-ranging in areas close to human territories across four provinces: Ratchaburi, Kanchanaburi, Lopburi, and Prachuap Khiri Khan. A few fecal samples from captive wild monkeys (a stump-tailed macaque, pig-tailed macaques, gibbons, and a leaf monkey) were also tested. Adenoviruses were detected in 33.3% (70 out of 210) of the fecal or rectal swab samples. The viruses identified in these samples included Simian adenovirus (SAdV)-A, SAdV-B, SAdV-H, Human adenovirus (HAdV)-D, HAdV-G, and a bat adenovirus species. One SAdV-B, SAdV RBR-7-10, was isolated from a long-tailed macaque fecal sample and identified by mass spectrometry. Its full hexon gene and nearly complete DNA polymerase gene were sequenced and analyzed, and the virions were imaged by transmission electron microscopy. The SAdV RBR-7-10 virus was used in a microneutralization assay to identify virus-specific antibodies in monkey plasma and human serum samples collected from the same areas in Prachuap Khiri Khan Province. We detected neutralizing antibodies against SAdV RBR-7-10 in 6.8% (n = 103) of the monkey samples but in none of the 125 human serum samples, suggesting no cross-species transmission of SAdV RBR-7-10 occurred at this study site. Nevertheless, a continuing surveillance of pathogens in monkeys is warranted to quickly identify possible emerging zoonotic outbreaks.

1. Introduction

Simian adenoviruses are non-enveloped, double-stranded DNA viruses of the genus Mastadenovirus in the family Adenoviridae, with icosahedral virions of 70–90 nm in diameter. Adenovirus genomes range from 26 to 48 Kbp in size and encode approximately 40 structural and non-structural proteins involved in host transcriptional modulation, viral DNA replication, virion assembly and maturation, and sabotage of
the host immune response. The *Adenoviridae* family is composed of six genera (*Atadenovirus*, *Aviadenovirus*, *Ictadenovirus*, *Mastadenovirus*, *Simadenovirus*, and *Testadenovirus*), and viruses in these groups infect mammals, birds, fish, reptiles, and amphibians [1]. Members of the genus *Mastadenovirus* infect a wide range of mammals, including humans, non-human primates (NHPs), and many others (cattle, dogs, deer, horses, pigs, sheep, rodents, bats, dolphins, sea lions, and polar bears). Adenovirus infections cause diseases that range widely in severity, from asymptomatic to fatal, in mammals. Clinical manifestations can include respiratory symptoms, gastroenteritis, conjunctivitis, meningitis, hepatitis, and systemic infection. A high mortality rate is associated with an immunosuppressive status and other host factors, especially in patients who are particularly young or old [2]. The classification of virus species within the genus depends on several characteristics, including genomic properties, serological properties, host range, and oncogenicity in rodents. Currently, the genus and species demarcation is based mainly on genomic criteria, i.e., phylogenetic distance, but also on genome organization and biological characteristics [1]. *Mastadenovirus* members currently include >50 species; among them are seven *Human mastadenovirus* (HADV-A to -G) and nine *Simian mastadenovirus* (SADV-A to -I) species [1]. Several adenoviruses isolated from NHPs are classified as HADV species. Regarding species denomination, a species containing adenoviruses isolated from NHPs is still named as a *Human mastadenovirus* if one of its members was isolated from humans [3]. For example, chimpanzee adenoviruses (ChAdVs) such as ChAd-63, SADV-23, SADV-24, and SADV-25, which have been used in the development of viral vectors for gene transfer and vaccine development, are phylogenetically grouped into HADV-E [4].

Simian adenoviruses identified from a variety of asymptomatic or diseased wild and captive NHPs, including the great apes (chimpanzees, gorillas, bonobos, and orangutans) [3,5–9], old-world monkeys (macaques, mustached monkeys, colobuses, and baboons) [3,7,10–15], and new-world monkeys [7,16,17] have been molecularly identified and characterized. Novel adenoviruses are continually characterized and added to both simian and human virus species. Owing to the genetic relatedness between monkey and human adenoviruses, interspecies transmission is anticipated [18]. To demonstrate the potential for cross-species transmission, phylogenetic and serologic evidence have been described in a number of publications. For example, a genome analysis-based evolutionary study suggested that HADV-B and HADV-E members originated in gorillas and chimpanzees, respectively [19]. These viruses have been zoonotically transmitted to humans in the past and are still circulating and causing diseases in humans [19]. The genetically identical nature of HADV-C viruses identified in humans and gorillas living in the same vicinity suggests that these viruses have also undergone interspecies transmission [3]. Genetic recombination was proposed as a mechanism involved in interspecies co-evolution and transmission [20,21]. Clinical and serological evidence of zoonotic transmission of viruses from monkeys to humans have been noted. As reported in two publications, researchers who were in close contact with colonies of titi monkeys and baboons, in which outbreaks of adenovirus-associated respiratory illnesses occurred, were seropositive for a tita monkey adenovirus and baboon adenovirus, respectively [12,17]. Flu-like symptoms were reported in the human contacts, and possible human-to-human transmission of the monkey-borne virus was indicated [17].

In Thailand, there are areas in which monkeys and humans live in close proximity, increasing the chances of human exposure to monkey viruses. Adenovirus transmission can occur through respiratory droplets, the fecal-oral route, the hand-ocular route, and fomites [18]. On the basis of the One Health concept, pathogen vigilance at the animal–human interface is one way to predict and detect an emerging zoonotic outbreak [22,23]. This study conducted a molecular survey performed by using PCR and nucleotide sequence analysis of adenovirus infection in free-ranging monkeys, mainly long-tailed macaques, and some captive monkeys (a stump-tailed macaque, pig-tailed macaques, gibbons, and a leaf monkey) in four Thai provinces: Ratchaburi, Kanchanaburi, Lopburi, and Prachup Khiri Khan. One simian adenovirus was isolated, characterized, and used in a micro-neutralization assay to detect virus-specific antibodies in humans and long-tailed macaques that share the same living area in Prachup Khiri Khan and predict the potential for cross-species transmission. Information about simian adenovirus infection in monkeys and humans is still limited, and we expect that our findings will help address this knowledge gap.

2. Materials and methods

2.1. Ethical statement

The animal study was conducted in accordance with the Ethical Principles and Guidelines for the Use of Animals for Scientific Purposes, the National Research Council of Thailand (NRCT). Our animal study protocol was reviewed and approved by the Animal Care and Use Committee of the Faculty of Tropical Medicine (FTM-ACUC), Mahidol University (No. FTM-ACUC 004/2022E). Our human study protocol was reviewed and approved by the Ethics Committee of the Faculty of Tropical Medicine, Mahidol University (No. MUTM 2022–008-01). The human serum samples used in this work were samples leftover from a previous study (EC approval number: MUTM 2019–058-01). During the consent process of that study, the participants had provided permission to use their leftover specimens for further studies.

2.2. Monkey sample collection and processing

Fecal (n = 120) and rectal swab (n = 90) samples were collected from 210 monkeys, specifically 194 long-tailed macaques (*Macaca fascicularis*), 1 stump-tailed macaque (*Macaca arctoides*), 8 pig-tailed macaques (*Macaca nemestrina*), 6 white-handed gibbons (*Hylobates lar*), and 1 leaf monkey (*Presbytis melalophos*), across four provinces, two in Central Thailand (Lopburi and Ratchaburi) and two in Western Thailand (Kanchanaburi and Prachup Khiri Khan), from 2013 to 2019.

The fecal samples were mixed with phosphate-buffered saline (PBS) to produce a 30% (wt/vol) fecal suspension. Each rectal swab, submerged in transport media, was mixed by vortexing and pressing the cotton swab to the wall of collection tube prior to discarding the cotton swab. All suspensions were centrifuged at 2000 × g for 10 min at 4 °C to pellet the fecal debris. The resulting supernatants were collected and stored at −80 °C until further use for RNA extraction or virus culture.

2.3. Nucleic extraction and polymerase chain reaction

Viral nucleic acid was extracted from 200 μl of processed fecal/rectal swab sample or virus culture supernatant with the GenUP™ Virus DNA/RNA kit (Biotechribbit GmbH, Berlin, Germany) in accordance with the manufacturer’s instructions. Nucleic acid was eluted in 60 μl of elution buffer and stored at −80 °C until further use.

For adenovirus molecular detection in fecal and rectal swab samples, nested PCR was performed on the extracted nucleic acid using primers specific to the DNA region encoding the polymerase gene (*Table 1*). The first-round PCR reaction contained 1× MyTaq HS Red Mix (Bioline, London, UK), 0.4 μM of each forward (Adeno-pol F1) and reverse (Adeno-pol R1) primer, and 5 μl of DNA template in a total volume of 25 μl. The thermal cycling conditions were: 95 °C for 3 min, followed by 35 cycles of 95 °C for 15 s, 55 °C for 15 s, and 72 °C for 90 s, and a final elongation at 72 °C for 10 min. The nested PCR reaction was prepared with primers Adeno-pol F2 and Adeno-pol R2 using 1 μl of the first-round PCR product. The thermal cycling conditions were: 95 °C for 3 min, followed by 35 cycles of 95 °C for 15 s, 55 °C for 15 s, and 72 °C for 30 s, and a final elongation at 72 °C for 10 min. PCR products were resolved on a 1.5% agarose gel, stained with gel red, and visualized under a gel documentation system (Molecular Imager Gel Doc XR imaging system, Bio-Rad, Hercules, CA, USA).
For adenovirus hexon and DNA polymerase gene amplification, PCR was performed with MyTaq HS Red Mix as described above, using 1 μl of the nucleic acid extracted from a virus culture supernatant and sets of overlapping primers specific to the region in the hexon (Hex18078F/Hex18078R, Adeno-hex F/Adeno-hex R, and Hex20855F/Hex20855R) and DNA polymerase (Pol5030F/Pol5030R, Adeno-pol F1/Adeno-pol R1, Pol8626F1/Pol8626R1, and Pol8626F2/Pol8626R2) genes (Table 1). The thermal cycling conditions were as described above, except that the length of time for the elongation at 72 °C was varied as follows: 120 s for primer set Adeno-hex F/Adeno-hex R; 90 s for primer set Adeno-pol F1/Adeno-pol R1; 60 s for primer set Hex18078F/Hex18078R and Pol5030F/Pol5030R.

2.4. Nucleotide sequence analysis

For the sequencing of a partial DNA polymerase gene from monkey samples, nested PCR products (258 bp) were excised from the agarose gel, and the DNA was purified using the QiAquick gel extraction kit (Qiagen, Hilden, Germany) in accordance with the manufacturer’s instructions. Purified DNA was sent for Sanger sequencing at Bionics Co., Tokyo, Japan using Primer Sequence (5'-3') Region Position Size (bp)

| Primer         | Sequence (5'-3') | Region      | Position | Size (bp) |
|----------------|------------------|-------------|----------|-----------|
| Adeno-pol F1   | TGATGCGGTTCTTACCTTGCCTGTTACCAG | DNA polymerase | 5524-5554 | 1515      |
| Adeno-pol R1   | AGTGYTACATGGTGGCTCTTAAGGG | DNA polymerase | 7014-7038 | 258       |
| Adeno-pol F2   | GTGACAAGAGGGCTGCGTGCTGCCACTGTA | DNA polymerase | 5571-5599 | 258       |
| Adeno-pol R2   | TAATGTCGCGCTTCTGCTGCTGCCACTGTA | DNA polymerase | 5799-5828 | 258       |
| Pol5030F      | AGAAGAGCGCTGCGGCGAGGT  | DNA polymerase | 6568-5078 | 1140      |
| Pol5030R      | CTTGCTCTCAGATTGCTGCCAGCCAATCAC | DNA polymerase | 6896-6915 | 905       |
| Pol8626F1     | GGTCTCGGGATGAGGCTGCTGCCACTGTA | DNA polymerase | 7779-7800 | 905       |
| Pol8626F2     | GGCTCGGGGCTAGAGGCTGCTGCCACTGTA | DNA polymerase | 7602-7620 | 905       |
| Pol8626R2     | TCGAGCTACCTGCGGCGAGGT  | DNA polymerase | 8720-8741 | 905       |
| Hex18078F     | GGCGAGAGCGCTAAGACAT | pVII | 17,935-17,995 | 385       |
| Hex18078R     | TTTAGAAGATGCCGGGCTGCCACTGTA | Hexon | 18,299-18,319 | 1140      |
| Hex20855F     | CAGGATGCTTCGGAGTACCTTGCTGCCACTGTA | Hexon | 18,129-18,151 | 1140      |
| Hex20855R     | AGTTYTACATGCTGGGCTCTTACCG | DNA polymerase | 19,779-19,844 | 1140      |
| Hex20855F     | AGCGGCTCTTTCGCTGCCACTGTA | DNA polymerase | 19,947-19,972 | 1140      |
| Hex20855R     | CAGGATGCTTCGGAGTACCTTGCTGCCACTGTA | DNA polymerase | 19,779-19,799 | 1140      |
| Hex20855R     | GGGAAGAGGGCTTGGCTGAAGT | Protease | 20,925-20,945 | 1140      |

2.5. Virus isolation

Virus isolation was performed using Vero cells (ATCC CCL81). The cells were grown in Dulbecco’s modified Eagle medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 1× Antibiotic-Antimycotic solution, and 1× GlutaMAX (Gibco™, Thermo Fisher Scientific, Waltham, MA, USA) to a confluence of 80%-90%. The supernatants of 30% (wt/vol) fecal solutions were filtered through a 0.45 μm pore-sized membrane using a syringe filter and then diluted five-fold with viral growth medium (DMEM supplemented with 2% FBS, 1× Antibiotic-Antimycotic solution, and 1× GlutaMAX). The Vero cell monolayer in a 96-well tissue culture plate was inoculated with 50 μl/well of the diluted filtered samples (3 wells/sample). Virus adsorption was achieved by centrifugation of the plate at 25 °C for 20 min prior to incubation at 37 °C under a 5% CO₂ atmosphere for 20 min. The centrifugation step was repeated before viral growth medium (150 μl/well) was added, and the cells were incubated at 37 °C under a 5% CO₂ atmosphere. The cells were observed microscopically for cytopathic effect (CPE) daily for 5 days. The supernatants and cells from CPE-positive wells were collected and stored at −80 °C. Two blind passages were performed.

2.6. Viral protein identification by mass spectrometry

The supernatant (50 ml) from virus cultured in Vero cells was collected, and cell debris was removed by centrifugation at 1000 × g for 15 min at 4 °C. The viruses in the supernatant were concentrated by ultracentrifugation in polycarbonate centrifuge bottles (no. 355603, Beckman Coulter, Brea, CA, USA) using a Beckman L7-65 ultracentrifuge (rotor 70.1 Ti) set at 35,000 rpm for 1.5 h at 4 °C. The resulting pellets were mixed with lysis buffer containing 1% NaCl, 1% sodium dodecyl sulfate (SDS), and 1% Triton-X to produce a virus protein lysate, which was then processed for mass spectrometric analysis via LC-MS/MS by a MicroToF Q II mass spectrometer (Bruker, Germany) coupled to an Ultimate 3000 nano- LC system (Dionex, Sunnyvale, CA, USA) as previously described [24].

2.7. Hemagglutination assay

Hemagglutination assays were performed as previously described [25]. Equal amounts of two-fold serially diluted virus and red blood cells (RBCs) were mixed and incubated at room temperature. Goose and chicken RBCs were used at a concentration of 0.5%, whereas sheep and human RBCs were used at a concentration of 0.75%.

2.8. Transmission electron microscopy

Virus-infected Vero cells were collected by centrifugation at 1000 × g for 15 min at 4 °C. The resulting cell pellets were fixed with 2.5% glutaraldehyde for 1 h and subsequently fixed with 1% osmium tetroxide for 1 h, at room temperature. The fixed pellets were dehydrated with a graded ethanol series, infiltrated and embedded in LR white resin
Numbers of monkey samples that tested positive for adenovirus by nested PCR.

| Site            | Sample type | Collection year | Species            | No. of samples | No. of positive (%) |
|-----------------|-------------|-----------------|--------------------|----------------|---------------------|
| Ratchaburi      | Feces       | 2018-2019       | M. fascicularis    | 38             | 11 (28.9%)          |
|                 |             |                 | M. arctoides       | 1              | 1                   |
|                 |             |                 | M. nemesrina       | 8              | 0                   |
|                 |             |                 | H. lar             | 6              | 1 (16.7%)           |
| Kanchanaburi    | Feces       | 2018-2019       | P. melalophos      | 1              | 1                   |
|                 |             |                 | M. fascicularis    | 12             | 4 (33.3%)           |
| Lopburi         | Feces       | 2013            | M. fascicularis    | 54             | 15 (27.8%)          |
| Prachuap Khiri Khan | Rectal swab | 2017           | M. fascicularis    | 90             | 37 (41.1%)          |
|                 |             |                 |                    | 70             | 70 (33.3%)          |
Fig. 1. Phylogenetic analysis of partial DNA polymerase nucleotide sequences (227 bp) from monkey samples. The unrooted tree was constructed using the maximum likelihood method with a bootstrap value of 1000. The accession numbers of the sequences obtained in this study and of the reference sequences are shown. Bootstrap values of ≥75 are shown at the node. The bar represents nucleotide substitutions per site. The color and symbol labeling used for collection sites (provinces) and monkey types, respectively, are indicated in the figure. Asterisks (*) indicate samples in which an adenovirus was isolated in Vero cell culture.
Thailand is home to many species of monkeys living in the wild or free-ranging near human territories. In the provinces where our samples were collected, monkeys are present in public areas, e.g., temples or historic sites, where they share the space with locals and travelers and thereby pose a risk of exposing humans to monkey pathogens. The majority of these monkeys are long-tailed or cynomolgus macaques (*M. fascicularis*), which are old-world monkeys endemic to Thailand and other Southeast Asian countries such as Myanmar, Malaysia, The Philippines, and Indonesia. Regarding adenovirus surveillance in monkeys, many reported molecular detections and phylogenetic characterizations of such viruses are from African countries, home to several great apes, old-world monkeys, and new-world monkeys [3,5,6,9]. Molecular detection of monkey adenovirus is generally conducted with animal stool samples, or occasionally with organs or blood, using PCR targeting the DNA polymerase and hexon genes. Detected prevalence greatly varies among species and settings. For example, in great apes (chimpanzees, gorillas, and bonobos), the adenovirus prevalence ranges from 36%–100% [3,5,6,9]. Unlike great apes, which are native to only Africa, different species of macaques (*Macaca* species) are distributed in both Africa and Asia. In addition to living in the wild, colonies of macaques are also present in wildlife sanctuaries, and some, mostly rhesus macaques, are kept for research purposes. An adenovirus survey in non-symptomatic macaques (*Macaca mulatta, M. fascicularis, and Macaca sylvanus*) from the US, China, and Africa revealed prevalences ranging from 13%–100% [3,9,14]. Limited adenovirus prevalence data from Asia is available, and there is no reported adenovirus surveillance of free-ranging long-tailed macaques in Thailand. The only adenovirus surveillance reported from Thailand was conducted in *Macaca assamensis* in a wildlife sanctuary; it detected a prevalence of 5.7% [26]. Here, molecular detection revealed an adenovirus prevalence of 33.3%. All but three of our adenovirus-positive samples were from free-ranging long-tailed macaques; the rest were from a captive wild stump-tailed macaque, a leaf monkey, and a white-handed gibbon, all species found in Thailand forests. The data suggest that adenoviruses naturally circulate in monkeys, with different prevalences among various species and locations.

The adenoviruses detected in long-tailed macaques here were tentatively grouped into SAdV-A, SAdV-B, SAdV-H, and HAdV-G species on the basis of their partial DNA polymerase sequences. Macaques are a source of SAdV-A and SAdV-B, which occasionally cause outbreaks of diarrheal and respiratory diseases in monkeys [9,12–14]. Here, SAdV-B was detected in all sampling locations, whereas SAdV-A was detected in three out of four locations; this difference could be due to the small sample size. SAdV-H-like sequences were detected in a few samples from Prachuap Khiri Khan. Members of SAdV-H, recently added to the group of Simian adenovirus species, include an adenovirus isolated from the
urine of a simian immunodeficiency virus (SIV)-infected rhesus macaque [27]. Overall, our results suggest that SAdV-A and SAdV-B are endemic to macaques in Thailand. Notably, there was no reported outbreak of disease among monkeys at the time of sample collection, and the monkey colonies assessed were non-symptomatic.

HAdV-G was also detected in all sampling sites, in a higher proportion compared with other adenovirus species, and in the one stump-tailed macaque included in our study. In contrast with HAdV-B, HAdV-C, and HAdV-E, which are widely detected in great apes, HAdV-G is prevalent in old-world monkeys [7]. An adenovirus survey in Assamese macaques in Northeast Thailand found HAdV-G as well [26]. In China, this virus was associated with diarrhea in captive macaques [13]. HAdV-G members include mainly simian adenoviruses identified in macaques. The only well-described human adenovirus is HAdV-52, associated with gastroenteritis in human hosts [28]. HAdV-G likely originated in monkeys and spread to humans via cross-species transmission from old-world monkeys [27]. Although HAdV-E was previously detected in Barbary macaques and other monkeys and apes in Africa [3], it was not detected here. Several studies have suggested a low host species specificity and consequent cross-species transmission potential.

**Fig. 4.** Transmission electron micrographs of SAdV RBR-7-10-infected Vero cells. Vero cells were infected with SAdV RBR-7-10 for two days and then subjected to transmission electron microscopy (TEM). (A) Electron micrograph of a virus-infected Vero cell at day 1 post-infection. Black arrows indicate the nuclear membrane. The area in the dashed box is enlarged and shown in (B), with blue arrows pointing to viral particles. (C) Electron micrograph of a virus-infected Vero cell at day 2 post-infection. Black arrows indicate the nuclear border. Red arrows point towards clumping of the nuclear chromatin. The area in the dashed box is enlarged and shown in (D), with black arrows indicating the nuclear border and blue arrows pointing to viral particles. (E–F) TEM images showing the non-enveloped icosahedral particles of SAdV RBR-7-10. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)
of HAdV-E from chimpanzees to African people [19]. Cross-species transmission may occur via close contact, contamination with body secretions, or during hunting. Our detection of a high prevalence of HAdV-G, rather than of HAdV-E, in macaques from different sites in Thailand demonstrates the importance of continuing surveillance of this virus species for its zoonosis potential in macaque-endemic areas.

HAdV-D-related adenoviruses were detected in a gibbon and a leaf monkey. HAdV-D may have originated in, and may be specific to, humans; it is isolated mainly from them [3,7,19]. The high levels of sequence identity between human and chimpanzee HAdV-D isolates in Africa may indicate cross-species transmission from humans to chimpanzees [7]. The two animals in our study from which HAdV-D was detected were captured from a forest, and there was some chance of animal contact with humans during their detainment. However, the sequence identities of these two HAdV-D-like viruses with published HAdV-D sequences were < 80%. Therefore, these viruses detected in gibbon and leaf monkey feces might be a HAdV-D variant transmitted from humans to the captive monkeys or they could belong to a new simian virus species in need of characterization.

This study also detected a bat adenovirus-like sequence, in a monkey fecal sample from Lopburi. Notably, we previously found a novel bat reovirus in monkey feces from the same site [24], thus confirming that, in areas where monkeys and bats cohabitate, there is a chance of virus cross-species transmission. Additional studies and evidence are needed to determine whether these results are from cross-species transmission or a cross-contamination of bat virus in the monkey specimen. Nevertheless, all currently available data support conducting zoonotic virus surveillance in monkeys and humans because previous reports and molecular surveys clearly demonstrate that various adenoviruses exist in areas where monkeys are endemic and a number of them have zoonotic potential. In addition, the lack of antibodies against SAdV RBR-7-10 in humans suggests that this virus could have potential utility as a viral vector. Adenoviral vectors have displayed their usefulness for gene transfer in vaccine development against HIV, malaria, and other pathogens [31,33,34]. The rising vector is pre-existing immunity against the vector in humans, which compromises vaccine efficacy [31]. Simian adenoviruses, such as chimpanzee adenoviruses, have become a target for vector development owing to their expected low levels of cross-immunity in humans. As such, SAdV RBR-7-10, with its low pre-existing immunity in humans, could be a candidate viral vector, although further study regarding this possibility is needed.

There are a few limitations of this study. First, its small sample size may be inadequate for making definitive conclusions regarding the spillover of SAdV-B at the human–animal interface. Second, the study samples were collected from only four study sites, which may not represent the epidemiology of SAdV-B for the entire country. However, the low SAdV-RBR-7-10 seropositivity in monkeys suggests it is unlikely that antibodies against this virus would be detected in the human population, even in individuals from a community in close proximity to monkeys. Nevertheless, the continuous monitoring of viruses in monkeys living in proximity to human populations is crucial for implementing the One Health approach to confronting and reemerging zoonotic disease threats.

5. Conclusions

While data of adenovirus survey in monkeys in Southeast Asia is limited, we reported the circulation of adenoviruses in monkeys, mainly long-tailed macaques in the central and western part of Thailand. SAdV-A, SAdV-B and HAdV-G were found in a high prevalence whereas SAdV-H, HAdV-D and bat adenovirus could be detected only in a few monkeys. Antibodies against SAdV-B were surveyed in monkeys and humans in the Prachup Khiri Khan Province and cross-species transmission was not observed in this study. However, since monkeys are considered a source of zoonotic pathogens, and the types of adenoviruses detected in this study have zoonotic potential, surveillance in monkeys and humans should be continued in more areas by collecting more samples from different monkey species; especially in areas where humans and monkeys share habitats.
monkeys live at the interface. The information concerning pathogens that may spillover from animals to human living in a close contact environment is important. It can be used to plan an emerging zoonotic disease control programs following the One Health concept.

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CRedit authorship contribution statement

Nathamon Kosoltanapiwat: Conceptualization, Validation, Formal analysis, Investigation, Resources, Writing – original draft, Writing – review & editing. Sumate Amapwong: Formal analysis, Investigation, Writing – review & editing. Luxsana Prasittichai: Investigation, Writing – review & editing. Narongchon Yingdee: Investigation, Writing – review & editing. Daraka Tongthainan: Resources, Writing – review & editing. Phitsanu Tulayakul: Resources, Writing – review & editing. Kopborn Boonnak: Conceptualization, Validation, Formal analysis, Resources, Writing – review & editing. Supervision, Funding acquisition.

Declaration of Competing Interest

None declared.

Data availability

Data will be made available on request.

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