1	Novel Encephalomyelitis-Associated Astrovirus in a Muskox (Ovibos moschatus) – a Surprise from the
2	Archives

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Abstract

Background: The small, single-stranded positive-sense RNA astroviruses are mostly known to be enteric viruses. In recent years, though, different astroviruses were reported in association with neurological disease in various species. In cattle, two distinct neurotropic astrovirus genotype species were described in numerous cases of nonsuppurative encephalomyelitis, with one of these viruses also reported in similar circumstances in several sheep. Here, we retrieved archived formalin-fixed, paraffin-embedded brain tissues of a muskox diagnosed with a comparable disease pattern in 1982 (ID 15375) and investigated them for the presence of neurotropic astroviruses with various techniques.

Results: Initially, tissue samples scored positive for both neurotropic astroviruses by immunohistochemistry; however, unexpected results with further immunohistochemical testing, *in situ* hybridization and qRT-PCR prompted us to submit an RNA extract from the animal's brain material to next-generation sequencing. We were thus able to obtain the full genome of a novel astrovirus, muskox astrovirus CH18 (MOxAstV-CH18), whose closest relative is an enteric ovine astrovirus. Subsequently, viral RNA could be detected with a specific RT-PCR in the brain of the affected animal, but not in faecal samples from the current muskoxen herd of the animal park where animal 15375 was kept.

Conclusions: We identified a novel astrovirus in a historical case of a captive muskox with nonsuppurative encephalomyelitis. Unfortunately, our results and the fact that no material from organs other than of the

36 nervous system was available do not allow any assumption about the epidemiology or pathogenesis of the

- 37 virus. Still, these findings are yet another piece of evidence that the tropism and species specificity of
- astroviruses could be more deceptive than generally assumed.
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Key words: Astrovirus, Encephalitis, Formalin-fixed and paraffin embedded (FFPE), Muskox (*Ovibos moschatus*), Next-generation sequencing (NGS).

42

43 Background

44 Muskoxen (*Ovibos moschatus*) are animals native to Arctic regions and belonging to the family *Bovidae*,

- 45 subfamily *Caprinae*. Although this species is not so common in captivity (115 animals registered in the
- 46 General ZIMS database as of January 29, 2019 [1]), small herds are kept in some animal parks (Fig. 1). Over
- 47 the last decades, substantial effort was put into the investigation of infectious diseases of free-ranging
- 48 muskoxen. For instance, parasites infesting these animals were described in numerous studies [2], and
- 49 several reports about specific outbreaks are available [3-6]. Finally, factors contributing to morbidity and
- 50 mortality in a declining population of Alaskan muskoxen were investigated in a comprehensive manner [7].
- 51 However, the knowledge about neurological diseases of these animals remains limited.
- 52 Astroviruses are small, nonenveloped viruses with a genome consisting of single-stranded, positive-sense
- 53 RNA. The latter includes three overlapping open reading frames (ORF) flanked by untranslated regions and
- 54 a poly-A tail, with ORF1a and ORF1b encoding nonstructural proteins (either as nsp1a or nsp1ab through a
- ribosomal frameshift mechanism) and ORF2 the capsid protein precursor [8]. Within the family Astroviridae,
- 56 members of the genus *Avastrovirus* infect birds, whereas those of the genus *Mamastrovirus* are found in
- 57 mammals. The taxonomy of astroviruses is currently based on their host species as well as their full capsid
- 58 protein precursor sequence, with amino acid distances (p-dist) greater than 0.338 defining distinct genotype
- 59 species [9]. Innumerable strains of these viruses have been described from faecal samples of various
- 60 mammalian species [10]. Apart from humans and minks, in which they are known to cause gastroenteric
- disease [8, 11], their association with illness in many animals yet remains unclear. In cattle and sheep,
- 62 astroviruses were found in diarrheic [12, 13] as well as healthy animals [14, 15]. Besides, astroviruses were
- 63 reported in association with neurological disease in an increasing number of hosts in recent years: humans
- 64 [16], minks [17], cattle [18], sheep [19] and pigs [20, 21]. Interestingly, many of these neurotropic
- astroviruses genetically cluster together in the so-called human-mink-ovine (HMO) clade, of which various
- 66 enterotropic strains are also part [20].
- 67 In cattle, two genotype species have been found in cases of nonsuppurative encephalitis: bovine astrovirus
- 68 CH13/NeuroS1 (BoAstV-CH13/NeuroS1) on the one hand and bovine astrovirus CH15/BH89-14 (BoAstV-
- 69 CH15/BH89-14) on the other. BoAstV-CH13/NeuroS1 [22] was reported from the USA [18], Switzerland
- [23], the UK [24], Canada [25, 26] and Japan [27], and could be detected in around one quarter of the cases

71 investigated in retrospective studies [18, 23, 25, 28, 29]. In contrast, BoAstV-CH15/BH89-14 was described

- in only three cattle up to date [30, 31]. Interestingly, viruses almost identical to this second astrovirus
- 73 genotype species were reported in several neurologically diseased sheep: they were denominated ovine
- astrovirus UK/2013/ewe/lib01454 and UK/2014/lamb/lib01455 in two sheep from the UK [19] and ovine
- astrovirus CH16 (OvAstV-CH16) [32] and CH17 (OvAstV-CH17) [33] in two Swiss cases. Being
- 76 genetically highly similar to one another, this group of neurotropic astroviruses of cattle and sheep are
- considered to belong to the same genotype species and will be referred to here as BoAstV-CH15/OvAstV-
- 78 CH16.
- 79 Since their discovery, we developed several diagnostic tools in order to study these bovine and ovine
- 80 neutropic astroviruses: *in situ* hybridization (ISH) [23], immunohistochemistry (IHC) [32, 34] and qRT-PCR
- 81 [35]. Recently, we were told about a historical case of a captive muskox that was diagnosed with
- 82 nonsuppurative encephalomyelitis in our division in 1982 (Prof. M. Vandevelde, personal communication),
- and were therefore curious whether astroviruses also played a role in this case. We retrieved formalin-fixed,
- 84 paraffin-embedded (FFPE) central nervous system tissue samples of this animal from our archive and
- 85 investigated these by IHC and ISH for the presence of BoAstV-CH13/NeuroS1 and BoAstV-CH15/OvAstV-
- 86 CH16. We then extracted RNA from the animal's brain tissue and performed qRT-PCR as well as next-
- 87 generation sequencing (NGS) on it. This lead to the discovery of a novel astrovirus.
- 88

89 **Results**

90 Affected animal

- 91 In January 1982, a six-year old male muskox (Ovibos moschatus, animal ID 15375) kept at Berne Animal
- 92 Park (Bern, Switzerland) suddenly showed weakness of the hind limbs, which rapidly progressed to
- 93 tetraplegia. After six days of supportive care without clinical improvement, the animal was released from
- 94 suffering by a chest hit. Central nervous system tissues were subsequently submitted to diagnostic
- 95 neuropathological investigation. Histopathologically, all segments of the spinal cord examined as well as the
- 96 midbrain displayed strong nonsuppurative lesions, particularly in the grey matter, with perivascular cuffs,
- 97 neuronal degeneration and gliosis (Fig.2). Although this lesion pattern is indicative of a viral infection, no
- 98 etiological diagnosis could be pinpointed at that time.
- 99 *IHC*
- 100 Three decades later, we retrieved FFPE central nervous system samples (midbrain and spinal cord) of animal
- 101 15375 from our archives. When testing this material for the presence of capsid antigen of BoAstV-
- 102 CH13/NeuroS1 and BoAstV-CH15/OvAstV-CH16 with a first hyperimmune serum each (CH13-ORF2-con
- 103 [34] and CH15-ORF2-var [32], respectively), positive staining was obtained for both viruses in all regions
- 104 investigated (Fig. 3, panels a and b). Subsequently, in an attempt to confirm our findings, we used a second
- 105 hyperimmune serum for each virus (CH13-23917 and CH15-ORF2-con [32], respectively), and obtained

- 106 discrepant results: negative staining for BoAstV-CH13/NeuroS1 (conversely, our index case for BoAstV-
- 107 CH13/NeuroS1, cow 45664 [23], reacted positively), contrasting with a distinctly positive one for BoAstV-
- 108 CH15/OvAstV-CH16 (Fig. 3, panels c and d). Brain tissue sections of two other muskoxen without
- 109 pathological lesions were negative with all antibodies.
- 110 **ISH**
- 111 We then tested all available brain regions of muskox 15375 with a dual ISH protocol for the detection of
- 112 BoAstV-CH13/NeuroS1 and BoAstV-CH15/OvAstV-CH16. Whereas our BoAstV-CH13/NeuroS1 [35] and
- 113 BoAstV-CH15/OvAstV-CH16 [32] controls both reacted positively for the individual viruses, all muskox
- 114 samples remained negative.
- 115 *qRT-PCR for bovine and ovine neurotropic astroviruses*
- 116 As the strongest staining in IHC was observed in the midbrain of animal 15375, we extracted RNA from this
- brain region. We investigated this purified RNA with two qRT-PCR protocols, one specific for BoAstV-
- 118 CH13/NeuroS1 [35], the other for BoAstV-CH15/OvAstV-CH16; both scored negative.
- 119 NGS and sequence analysis
- 120 Despite the inconclusive results obtained by qRT-PCR, we chose to submit the RNA extract from muskox
- 121 15375's midbrain to NGS. As RNA from FFPE tissue, especially if old, can be expected to be strongly
- fragmented, we sequenced 100 bp-long reads in single-end mode, and obtained 198'031'783 of them. After
- quality-trimming, 186'002'398 reads were used for assembly. Three contiguous sequences (contigs) \geq 500 nt
- 124 long and displaying a similarity to astroviruses on nucleotide and/or amino acid level were generated and
- 125 finally reassembled. The complete sequence obtained (GenBank accession no. MK211323.1) was 6515 nt
- long, with a series of adenines at the 3' end corresponding to the virus's polyadenylated tail. No RACE was
- 127 carried out to determine the exact ends of the viral genome. The genome contained three putative
- 128 overlapping ORFs, with a characteristic ribosomal frameshifting signal at the ORF1a/ORF1b junction.
- 129 ORF1ab displayed 97.6% (resp. 87.8%) and ORF2 74.6% (resp. 70.5%) amino acid (resp. nucleotide)
- 130 identity to their best hits, which were both on ovine astrovirus 1 (OvAstV-1, that was isolated from the
- 131 faeces of diarrheic lambs [12, 36]; GenBank accession number NC_002469.1). Phylogenetic analyses based
- 132 on capsid protein precursor and nonstructural polyprotein sequences confirmed that the closest relative of the
- 133 novel astrovirus is OvAstV-1 (Fig. 4). The p-dist between the capsid protein precursor of these viruses is
- 134 0.257, which classifies them as the same genotype species according to the present standards of the
- 135 International Committee on Taxonomy of Viruses [9]. Finally, other bovine and ovine neurotropic
- 136 astroviruses also clustered in the same branch of the phylogenetic tree.
- 137 **RT-PCR** for muskox astrovirus
- 138 We designed RT-PCR primers based on the sequence of the novel astrovirus obtained by NGS and our
- 139 bioinformatics pipeline. The RNA extract from FFPE midbrain tissue of muskox 15375 used for NGS
- 140 produced an amplicon of the expected size (108 bp). Besides, as most mamastroviruses are enteric viruses,

141 we wondered whether the novel astrovirus is to be commonly found in muskoxen's faeces. However, RNA

142 extracted from faecal samples of the five current muskoxen herd members of the zoo where muskox 15375

143 was kept 30 years ago remained negative for the virus.

144

145 **Discussion**

146 We report a novel astrovirus, discovered in association with a case of nonsuppurative encephalomyelitis in a

147 captive muskox (Ovibos moschatus) that was sacrificed in 1982 because of neurological symptoms (ID

148 15375). After initial immunohistochemical reactivity for two neurotropic astroviruses previously reported in

- cattle and sheep, contradictory outcomes of additional investigations prompted us to submit an RNA extract
- 150 from FFPE brain tissue of the animal to NGS. We thus obtained the full-length sequence of an astrovirus,
- 151 which we tentatively name muskox astrovirus CH18 (MOxAstV-CH18), and whose closest relative is an
- 152 ovine enteric astrovirus, OvAstV-1 [12, 36].
- 153 Cross-reactivity in our IHC assays could be explained by some degree of antigenic similarity between
- 154 MOxAstV-CH18 and bovine and ovine neurotropic astroviruses. Indeed, numerous stretches up to 37 amino
- acids in length are conserved among the capsid protein precursors of these viruses. For three of the
- polyclonal antisera we used in IHC, the viral antigens used to obtain them consisted of 313 to 373 amino
- acids; some of their epitopes are thus probably also present in the capsid protein of MOx-AstV-CH18.
- 158 Conversely, the amino acid sequence corresponding to a 16 amino acid-long peptide used to obtain some
- 159 BoAstV-CH13/NeuroS1-specific antibodies (CH13-23917) that reacted negatively in IHC is not found in
- 160 MOxAstV-CH18. Conversely, there is probably too much variation at nucleotide level for the dual ISH and
- both qRT-PCRs specific for BoAstV-CH13/NeuroS1 and BoAstV-CH15/OvAstV-CH16 to recognize
- 162 MOxAstV-CH18 in brain tissue samples of animal 15375.
- 163 As we did not have other muskoxen cases with comparable disease in our archive, we could not investigate
- 164 further whether MOxAstV-CH18 occurs regularly in such circumstances. Moreover, as we could not find
- 165 specific reports about neuroinfectious diseases in muskoxen in the literature, astrovirus-associated
- 166 encephalomyelitis is probably an exceptional finding in this species. Yet, in order to investigate whether
- 167 MOxAstV-CH18 is a common enteric virus of muskoxen, we tested by RT-PCR several faecal samples
- 168 obtained from the current herd of the animal park where muskox 15375 was kept, but all were negative. This
- 169 inconclusive finding therefore leaves open all speculations about the epidemiology and pathogenesis of the
- 170 virus. Still, the fact that the closest relative of MOxAstV-CH18 is an astrovirus that was isolated from
- 171 diarrheic lambs [12, 36] raises the question of inter-species transmission. Indeed, even though astroviruses
- are generally assumed to be host-specific, an increasing number of studies puts this assumption into question
- 173 [37-40]. Moreover, our results highlight the potential hazard that the proximity of sheep could represent to
- the health status of muskoxen populations. Sheep were already considered to be the most probable origin of

two epizootics in Norwegian muskoxen: one of contagious ecthyma (orf) [4] and one of pneumonia due to *Mycoplasma ovipneumoniae* [5].

177 Recently, an astrovirus was described from the faeces of a Sichuan takin (Budorcas taxicolor ssp. tibetana)

178 [41]. Takins belong to the subfamily *Caprinae*, as muskoxen do. Interestingly, phylogenetic analysis showed

that MOxAstV-CH18 genetically cluster together with an ovine faecal astrovirus, in a clade distant from that

180 of the takin astrovirus and bovine enteric counterparts. Differences in tropism might explain the genetic

- 181 divergence of the viruses.
- 182 Because of treatment with formalin, the integrity of nucleic acids extracted from FFPE tissues is generally
- assumed to be compromised, with fragmentation and cross-linking of molecules [42]. In cancer research,

184 however, FFPE tissue is increasingly considered a valuable source of nucleic acids to study [43]. Conversely,

- 185 the number of virological studies performed with such material is sparse, with relatively few studies using
- 186 NGS [44-47]. In that regard, the most prominent example is probably the determination, in one NGS run, of
- 187 the full genome of the 1918 pandemic influenza strain that previously took nine years to complete with
- traditional sequencing methods [48]. Yet, here we were able to recover the whole genome length of a novel
- astrovirus from FFPE brain tissue by NGS and *de novo* assembly. These results therefore demonstrate the
- 190 power of this approach, also in such conditions, and support its use for viral discovery in archived material as
- 191 well, highlighting the huge potential for retrospective investigations of unresolved cases or even epidemics.
- 192

193 Conclusions

194 Our data indicate that MOxAstV-CH18 is a possible cause of nonsuppurative encephalomyelitis in

195 muskoxen. This warrants further investigation into the spectrum of diseases (in particular of the nervous

196 system) affecting captive and wild muskoxen, as well as other ruminant species. We also show that NGS

197 enables straightforward virus discovery also when applied to FFPE tissues. Finally, the close phylogenetic

- 198 and antigenic relationships of MOxAstV-CH18 to other ruminant neurotropic astroviruses further question
- 199 the concept of a strict host specificity for this virus family.
- 200

201 Methods

202 Tissue samples

FFPE central nervous system tissues (midbrain and thoracic spinal cord) of a muskox (*Ovibos moschatus*, ID 15375) were available from the archive of the Division of Experimental Clinical Research, Vetsuisse Faculty, University of Bern (Bern, Switzerland). The animal was submitted to neuropathological investigation in 1982 after euthanasia because of progressive neurological disease unresponsive to therapy. Original tissues samples had to be re-embedded before further processing. Brain sections of two other

- 207 muskoxen without neuropathological lesions were also available from this archive.
- 208 Faecal samples
- 209 Individual faecal samples from all (five) members of the present muskoxen herd of Berne Animal Park were collected between June
- 210 and July 2018 and stored at 4 °C until further processing.
- 211 ІНС

212 Firstly, all brain regions available were screened with our usual IHC protocols for the presence of BoAstV-CH13/NeuroS1 (using

- 213 hyperimmune serum CH13-ORF2-con) and BoAstV-CH15/OvAstV-CH16 (using hyperimmune serum CH15-ORF2-var). Secondly,
- 214 samples were tested with other hyperimmune sera: one specific for BoAstV-CH13/NeuroS1 (CH13-23917) and one for BoAstV-
- 215 CH15/OvAstV-CH16 (CH15-ORF2-var). The generation procedures for hyperimmune sera CH13-ORF2-con, CH15-ORF2-var and
- 216 CH15-ORF2-con are described elsewhere [32, 34]. Polyclonal antibodies CH13-23917 were obtained by immunizing rabbits with a
- 217 short polypeptide derived from the capsid protein precursor sequence of our BoAstV-CH13/NeuroS1 index case [23] (ID 45564;
- 218 amino acids 60-73 of the capsid protein gene of Bovine astrovirus CH13, GenBank accession no. NC_024498.1). The immunization
- 219 and subsequent affinity purification of the hyperimmune serum were performed at BioGenes GmbH (Berlin, Germany). Regarding
- 220 the IHC method, tissue sections were first deparaffinised, rehydrated, and endogenous peroxidase activity was blocked in a solution
- 221 of 3% H₂O₂ in methanol. They were then microwave cooked in Dako Target Retrieval Solution, pH 9 (Dako Denmark A/S, Glostrup,
- 222 Denmark) for antibodies CH13-ORF2-con and CH13-23917, or Dako Target Retrieval Solution, Citrate pH 6 (Dako Denmark A/S,
- 223 Glostrup, Denmark) for antibodies CH15-ORF2-var and CH15-ORF2-con. Blocking was performed with 10% Goat Serum (Normal)
- 224 (Dako Denmark A/S, Glostrup, Denmark) in phosphate-buffer saline with 0.5% Tween (PBS-T). The samples were incubated with 225 each primary antibody CH13-ORF2-con (diluted 1:100 in PBS-T), CH13-23917 (diluted 1:50 in PBS-T), CH15-ORF2-var (diluted
- 226
- 1:50 in PBS-T) and CH15-ORF2-con (diluted 1:50 in PBS-T) overnight at 4 °C. Finally, detection was carried out with Dako REAL
- 227 Detection System (Dako Denmark A/S, Glostrup, Denmark), following the manufacturer's instructions.
- 228 ISH

229 The attempt to detect viral RNA in situ was carried out with the RNA Scope Assay [49]. A probe specific for BoAstV-CH15

- 230 (RNAScope Probe BoAstV-CH15-C2) was developed and used in combination with a probe specific for BoAstV-CH13/NeuroS1
- 231 (RNAScope Probe BovineAstrovirus, already commercially available) in the RNAscope 2.5 HD Duplex Detection Kit (Advanced
- 232 Cell Diagnostics, Newark, NJ), following the manufacturer's guidelines.

233 **RNA** extraction

- 234 RNA from FFPE material was extracted essentially as described in a study of Delnatte and colleagues [50]. Briefly, two 20 µm-thick 235 sections of FFPE midbrain of muskox 15375 were deparaffinised with xylol and further processed with the RNeasy FFPE kit 236 (Qiagen, Hilden, Germany) according to the manufacturer's instructions. All assays described below for animal 15375 were 237 performed with the same RNA extract. RNA from faeces was isolated using the QIAamp Viral RNA Mini kit (Qiagen, Hilden, 238 Germany). Faecal samples were first diluted in phosphate buffered saline to a concentration of 20% v/v, centrifuged for 20 min at 239 4'000 x g and 4 °C, and the supernatant was filtered through a 0.2 µm-filter before being purified according to the manufacturer's 240 instructions. The positive RNA controls used in this study were extracted with TRI Reagent (Sigma Life Science, St. Louis, MO) 241 from frozen brain tissue of one BoAstV-CH13/NeuroS1- (ID 26875) [35] and one OvAstV-CH16-case (ID 41669) [32]. 242 *qRT-PCR* for bovine and ovine neurotropic astroviruses
- 243 Three or one µL RNA extract from FFPE midbrain tissue of muskox 15375 or frozen brain tissue of animals 26875 and 41669, 244 respectively, were investigated for the presence of BoAstV-CH15/OvAstV-CH16 sequences with the AgPath-ID RT-PCR kit 245 (Ambion, Austin, TX) according to the manufacturer's instructions. The primer combination CH13-A [35] (targeting ORF1a of 246 BoAstV-CH13/NeuroS1) served for the detection of BoAstV-CH13/NeuroS1, whereas BoAstV-CH15/OvAstV-CH16 was tested 247 with the primer combination CH15 [33] (targeting ORF2 of BoAstV-CH15/OvAstV-CH16). Both assays were run on a 7300 Real 248 Time PCR system (Applied Biosystems, Singapore) with the following conditions: 45 °C for 10 min, 95 °C for 10 min, 40 cycles of 249 95 °C for 15 s, 62 °C for 20 s, 60 °C for 30 s.

250 NGS

- 251 Starting material for library preparation was 50 ng RNA extract from FFPE midbrain tissue of muskox 15375. cDNA synthesis was
- 252 performed without fragmentation with the SMARTer Stranded Total RNA-Seq Kit v2 - Pico Input Mammalian (Takara Bio USA,
- 253 Mountain View, CA), with repeated purifications with AMPure XP beads (Beckman Coulter, Brea, CA). Before single-end
- 254 sequencing (100 bp) on half a lane with a HiSeq 3000 System (Illumina, San Diego, CA), the library quality was controlled on a

- 255 Qubit Fluorometer (Life Technologies, Eugene, OR) and a Fragment Analyzer (Advanced Analytical Technologies, Ankeny, IA)
- with the High Sensitivity NGS Fragment Analysis Kit (Advanced Analytical Technologies, Ankeny, IA).

257 De novo assembly

- 258 Raw reads were quality-trimmed using trimmomatic (Ver. 0.36). As no reference genome is available for muskoxen (Ovibos
- 259 *moschatus*), quality-trimmed reads were assembled directly using SPAdes (Ver. 3.12.0). The generated contigs were aligned to viral
- databases with BLASTN (Ver. 2.7.1+, using viral sequences from Genbank and RefSeq downloaded on July 25, 2018) and
- 261 DIAMOND (Ver. 0.9.18, using viral sequences from UniProt downloaded on June 13, 2018) on nucleotide and amino acid level,
- respectively.

263 Phylogenetic analysis

- 264 Phylogenetic analysis was conducted on the amino acid sequence of the capsid protein precursor of the novel virus and 44
- 265 representative members of the family Astroviridae. For the phylogenetic analysis of the nonstructural polyprotein precursor nsp1ab, 9
- 266 representative members of the family Astroviridae were used in addition to the novel virus. All sequences were imported into MEGA
- 267 (Ver. 7.0.26) and aligned using the built-in MUSCLE alignment tool. Maximum-Likelihood trees were generated based on a matrix
- described by Le and Gascuel [51].

269 RT-PCR for muskox astrovirus

- 270 RT-PCR primers were designed with Geneious 10.1.3 [52] based on the novel astrovirus sequence, with forward primer
- 271 MOxAstV_F: GGCGGGCCATAGGACTATTC and reverse primer MOxAstV_R: CTTTGGGCATGCTGGAGAGA. One or four
- 272 μL RNA from FFPE midbrain of animal 15375 or muskoxen faecal samples, respectively, were tested using the OneTaq One-Step
- 273 RT-PCR Kit (New England Biolabs, Ipswich, MA) using the alternative protocol described by the manufacturer.
- 274

275 Abbreviations

- 276 BoAstV-CH13/NeuroS1: bovine astrovirus CH13/NeuroS1
- 277 BoAstV-CH15: bovine astrovirus CH15
- 278 FFPE: formalin-fixed, paraffin-embedded
- 279 IHC: immunohistochemistry
- 280 MOxAstV-CH18: muskox astrovirus CH18
- 281 NGS: next-generation sequencing
- 282 OvAstV-CH16: ovine astrovirus CH16
- 283 OvAstV-CH17: ovine astrovirus CH17
- 284

285 Declarations

286 Authors' contributions

- 287 CLB designed the research study, performed experiments, analysed the data and wrote the paper. MCK
- 288 performed the bioinformatics analysis. RVK, EKG and MMH performed experiments. SH provided the
- faecal samples and the picture for Fig. 1. TS designed the research study and edited the article. All authors
- 290 read and approved the final version of the manuscript. All authors have read and approved the final version
- 291 of the manuscript.
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- 298 Social and Preventive Medicine, Lausanne University Hospital, Switzerland) for proofreading the article.
- 299 **Competing interests**
- 300 The authors declare that they have no competing interests.
- 301 Availability of data and materials
- 302 The raw data generated by next-generation sequencing can be found in the European Nucleotide Archive
- 303 under accession number ERS3126950. The genome of MOxAstV-CH18 is available in GenBank under
- accession number MK211323.1.
- 305 **Consent for publication**
- 306 Not applicable.
- **Ethics approval**
- 308 All animals in this study were submitted to diagnostic neuropathological investigation after dying of sickness
- 309 or being euthanized because of it, and approval for this study was therefore not required as per the local
- 310 legislation.
- 311 **Prior publication**
- 312 Data were not published previously.
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- 318

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429 Figures



Figure 1. Muskoxen (*Ovibos moschatus*) at Berne Animal Park (Bern, Switzerland). Muskox cow with her calf in the spring of 2018.

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Figure 2. Histopathological lesions in the midbrain of muskox (*Ovibos moschatus*) 15375. Note the gliosis on the upper left and the perivascular cuff on the lower right. Haematoxylin and eosin stain.

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Figure 3. Immunohistochemistry (IHC) for BoAstV-CH13/NeuroS1and BoAstV-CH15/ OvAstV-CH16 in the midbrain of muskox (*Ovibos moschatus*) 15375. a IHC using hyperimmune antiserum CH13-ORF2-con showing positive staining. b IHC using hyperimmune antiserum CH15-ORF2-var showing positive staining. c IHC using hyperimmune antiserum CH13-23917 showing negative staining. d IHC using hyperimmune antiserum CH15-ORF2-con showing positive staining.

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Figure 4. Phylogenetic analysis. Maximum-Likelihood trees constructed with a the capsid protein precursor
and b the nonstructural polyprotein nsp1ab sequences of the new astrovirus strain MOxAstV-CH18 and
selected astroviruses. GenBank accession numbers are shown in brackets. Neurotropic strains are indicated

- 438 with rhombi. Scale bars illustrate p-distances. AvAstV, avian astrovirus; BoAstV, bovine astrovirus;
- 439 HuAstV, human astrovirus; MiAstV, mink astrovirus; OvAstV, ovine astrovirus; PoAstV, porcine astrovirus;
- 440 StAstV, Sichuan takin astrovirus.