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Tissue distribution of angiotensin-converting enzyme 2 (ACE2) receptor in wild animals with a focus on artiodactyls, mustelids and phocids

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ABSTRACT

Natural cases of zooanthroponotic transmission of SARS-CoV-2 to animals have been reported during the COVID-19 pandemic, including to free-ranging white-tailed deer (*Odocoileus virginianus*) in North America and farmed American mink (*Neovison vison*) on multiple continents. To understand the potential for angiotensin-converting enzyme 2 (ACE2)-mediated viral tropism we characterised the distribution of ACE2 receptors in the respiratory and intestinal tissues of a selection of wild and semi-domesticated mammals including artiodactyls (cervids, bovids, camelids, suids and hippopotamus), mustelid and phocid species using immunohistochemistry. Expression of the ACE2 receptor was detected in the bronchial or bronchiolar epithelium of several European and Asiatic deer species, Bactrian camel (*Camelus bactrianus*), European badger (*Meles meles*), stoat (*Mustela erminea*), hippopotamus (*Hippopotamus amphibious*), harbor seal (*Phoca vitulina*), and hooded seal (*Cystophora cristata*). Further receptor mapping in the nasal turbinates and trachea revealed sparse ACE2 receptor expression in the mucosal epithelial cells and occasional occurrence in the submucosal glandular epithelium of Western roe deer (*Capreolus capreolus*), moose (*Alces alces alces*), and alpaca (*Vicunga pacos*). Only the European badger and stoat expressed high levels of ACE2 receptor in the nasal mucosal epithelium, which could suggest high susceptibility to ACE2-mediated respiratory infection. Expression of ACE2 receptor in the intestinal cells was ubiquitous across multiple taxa examined. Our results demonstrate the potential for ACE2-mediated viral infection in a selection of wild mammals and highlight the intra-taxon variability of ACE2 receptor expression, which might influence host susceptibility and infection.

1. Introduction

Angiotensin-converting enzyme 2 (ACE2) is a membrane associated enzyme involved in blood pressure homeostasis [1] that is also a host receptor that allows binding and entry of coronaviruses including severe acute respiratory syndrome coronavirus (SARS-CoV), SARS-CoV-2 and SARS-like viruses [2–4]. The amino acid sequence of ACE2 is generally conserved across mammals, although with slight species-differences in

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key residues that could alter virus and host receptor interactions [5]. The anatomical distribution of the ACE2 receptor, however, varies widely across mammalian species and the ACE2 expression profile can be linked to different disease outcomes from SARS-CoV-2 infection in animals [6–8].

During the COVID-19 pandemic, there have been multiple instances where animals acquired SARS-CoV-2 infection from humans (zooanthroponosis). The most widespread detection of SARS-CoV-2 infection in free-living wildlife populations has been among white-tailed deer (WTD; *Odocoileus virginianus*) reported in North America, as predicted from the high binding affinity of ACE2 with SARS-CoV-2 [5] and several experimental studies [9–11]. Numerous field studies have revealed transmission of contemporaneous variants of SARS-CoV-2 to WTD and viral evolution within the wild deer population [12–16]. Targeted surveillance of deer in Europe has, however, not found any evidence of incursion into native European or introduced Asiatic deer populations [17,18]. Currently the susceptibility of deer species present in Europe to SARS-CoV-2 is unknown.

Zooanthroponotic infection has been reported most in farmed American mink (*Neovison vison*; hereafter mink) and companion animals such as domestic dogs (*Canis lupus familiaris*), cats (*Felis catus*) and ferrets (*Mustela furo*) [19–21]. Infection in farmed mink led to extensive culling in order to prevent potential emergence and transmission of virus variants which could impede public health measures [22–24]. In addition to captive ferrets and American mink, other mustelid species known to be susceptible to SARS-CoV-2 include the Eurasian otter (*Lutra lutra*), pine marten (*Martes martes*) and European badgers (*Meles meles*) [21,25–28]. Ferrets can develop upper respiratory tract infection and mink with both upper and lower respiratory tract infection, due to differences in ACE2 receptor distribution [6,7]. Previously we described the presence of the ACE2 receptor on the bronchiolar epithelium in the lung of the European badger [6] but the upper respiratory tract has not

Table 1
Immunohistochemical characterisation of ACE2 receptors in the lung and small intestine.

Family	Species	Country /Region Sampled	Free living (F), captive (C), domestic (D)	Sample size	Lung	Intestine
Cervidae	Barasingha (*Rucervus duvaucelii*)	UK	C	4	–	n/a
	Chinese water deer (*Hydropotes inermis*)	UK	C	1	–	n/a
	Common fallow deer (*Dama dama*)	UK	F	5	Bronchiolar epithelium, medium-sized vessels, type I pneumocytes	n/a
	Moose (*Alces alces alces*)	Norway	F	3	–	n/a
	Norwegian semi-domesticated reindeer (*Rangifer tarandus tarandus*)	Norway	C	2	–	Enterocytes
	Pere David's deer (*Elaphurus davidianus*)	UK	C	1	–	n/a
	Reeves's Muntjac (*Muntiacus reevesi*)	UK	C	6	Bronchiolar epithelium, medium-sized vessels, type I pneumocytes	n/a
	Svalbard wild reindeer (*Rangifer tarandus platyrhynchus*)	Norway	F	4	–	Enterocytes
	Western red deer (*Cervus elaphus*)	UK	F	2	Bronchiolar epithelium, type I pneumocytes	n/a
	Western roe deer (*Capreolus capreolus*)	UK, Norway, Sweden	F	8	Bronchiolar epithelium, type I pneumocytes	Enterocytes
Bovidae	American bison (*Bison bison*)	UK	C	1	–	n/a
	Scimitar-horned oryx (*Oryx dammah*)	UK	C	2	–	Enterocytes
	Domestic yak (*Bos grunniens*)	UK	C	1	–	Enterocytes
	Nilgai (*Boselaphus tragocamelus*)	UK	C	2	–	n/a
Camelidae	Alpaca (*Vicugna pacos*)	UK	D	2	–	Enterocytes
	Bactrian camel (*Camelus bactrianus*)	UK	C	2	Type I pneumocytes	Enterocytes
	Llama (*Lama glama*)	UK	D	1	–	Enterocytes
Suidae	Eurasian wild boar (*Sus scrofa*)	UK	C	3	–	Enterocytes
Hippopotamidae	Common hippopotamus (*Hippopotamus amphibius*)	UK	C	1	Bronchiolar epithelium	n/a
Mustelidae	Asian small-clawed otter (*Aonyx cinereus*)	UK	C	3	Bronchiolar epithelium	Enterocytes
	European badger (*Meles meles*)	UK	F	3	Bronchiolar epithelium	Enterocytes
	European pine marten (*Martes martes*)	Sweden	F	1	n/a	Enterocytes
	European polecat (*Mustela putorius*)	UK	F	3	–	n/a
	Stoat (*Mustela erminea*)	UK	F	5	Bronchial epithelium	Enterocytes
	Least weasel (*Mustela nivalis*)	UK	F	5	–	n/a
Erinaceidae	West European hedgehog (*Erinaceus europaeus*)	UK	F	3	–	Enterocytes
Sciuridae	Eurasian red squirrel (*Sciurus vulgaris*)	UK	F	5	Medium-sized vessels	n/a
Cercopithecidae	Crested macaque (*Macaca nigra*)	UK	C	2	Bronchiolar epithelium	n/a
Macropodidae	Red-necked wallaby (*Notamacropus rufogriseus*)	UK	C	2	–	n/a
Phocidae	Grey seal (*Halichoerus grypus*)	UK	F	2	Bronchial and bronchiolar epithelium	n/a
	Harbor seal (*Phoca vitulina*)	Norway, Sweden	F	3	Bronchiolar epithelium	Enterocytes
	Harp seal (*Pagophilus groenlandicus*)	Greenland	F	4	–	Enterocytes
	Hooded seal (*Cystophora cristata*)	Greenland	F	5	Bronchial epithelium	Enterocytes

- negative; n/a, not available.

been characterised to fully understand the extent of receptor expression and therefore the risk of respiratory exposure to SARS-CoV-2. In Europe, some wild mustelids, such as the European badger, may come into close direct or indirect proximity of humans particularly where urban and peri-urban populations exist or during wildlife management programs, thus raising the possibility of zooanthroponotic infection [19]. Improved understanding of the distribution of ACE2 receptors in the respiratory tract of wild mustelids would help predict species susceptibility to SARS-CoV-2.

Although no cases have been reported in phocids to date, *in silico* analysis of their ACE2 receptors has suggested they may be susceptible to SARS-CoV-2 [29]. Some pinnipeds are known to be susceptible to respiratory pathogens including influenza viruses and coronaviruses [30–34].

As there is limited knowledge on the ACE2 expression profile in tissues, which is often based on transcript detection [35], the current study aimed to expand our knowledge of ACE2 receptor distribution by microscopy evaluation of tissues from a range of wild animals with a particular focus on artiodactyl, mustelid and phocid species. The localisation and abundance of ACE2 receptor expression could have implications for the tropism, pathogenesis, and transmission of ACE2-mediated viral infection.

2. Materials and methods

2.1. Immunohistochemistry

Formalin-fixed paraffin-embedded tissues were obtained from histology archives held by the Animal and Plant Health Agency (UK), the Zoological Society of London, the Norwegian Veterinary Institute, and the National Veterinary Institute in Sweden (Table 1). Tissue samples had been collected as part of routine veterinary investigations, health surveillance or from hunting and pest control operations, and adhered to local wildlife legislation. Tissues from all available species were analysed. Animals with known disease or tissues in a poor state of preservation as determined by histology were excluded from the immunostaining and analysis to avoid artefacts that could impact on histological interpretation.

Immunohistochemistry for ACE2 was conducted as described previously [6]. Briefly, tissue sections were quenched for endogenous peroxidase and epitope unmasking was conducted using a pH 6 buffer (Fisher Scientific, VWR International; UK) at 100 °C. The sections were then incubated with either a rabbit polyclonal ACE2 primary antibody (Abcam ab15348; UK) or with a concentration-matched isotype antibody as a negative control, followed by secondary immunolabelling with rabbit-specific Envision+™ HRP-labelled polymer (Dako; Denmark) and visualisation using 3,3-diaminobenzidine tetrahydrochloride. Kidney sections from each species were used as internal positive controls to verify immunolabelling.

3. Results

3.1. Detection of ACE2 receptor in the lung and small intestines

In artiodactyls, ACE2 immunolabelling was detected on the apical aspect of the bronchiolar epithelium of fallow deer (*Dama dama*), muntjac (*Muntiacus reevesi*), western red (*Cervus elaphus*) and roe deer (*Capreolus capreolus*) (Table 1, Fig. 1). Immunolabelling of type I pneumocytes was observed in fallow deer, muntjac, roe deer (Fig. 1), red deer (Fig. 3), and occasionally on the endothelium of medium-sized arterioles was immunolabelled in muntjac and fallow deer. Of the camelids examined, ACE2 was only detected in type I pneumocytes in the Bactrian camel (*Camelus bactrianus*) (Fig. 2) but not detected in the lung tissue of llama (*Lama glama)* or alpaca (*Vicugna pacos*) (Fig. 1). ACE2 was also detected on the bronchiolar epithelium of the hippopotamus (*Hippopotamus amphibius*). No ACE2 immunolabelling was observed in the lung sections of the American bison (*Bison bison*), barasingha (*Rucervus duvaucelii*), Chinese water deer (*Hydropotes inermis*), Pere David's deer (*Elaphurus davidianus*), moose (*Alces alces alces*), nilgai (*Boselaphus tragocamelus*), Norwegian semi-domesticated reindeer (*Rangifer tarandus tarandus*), Svalbard wild reindeer (*Rangifer tarandus platyrhynchus*), yak (*Bos grunniens*), scimitar-horned oryx (*Oryx dammah*) and wild boar (*Sus scrofa*).

Among the mustelids, the European badger (*Meles meles*; Fig. 1) and Asian small-clawed otter (*Aonyx cinereus;* Fig. 2) expressed ACE2 on the bronchiolar epithelium, whereas ACE2 was only detected in the bronchial epithelium of the stoat (*Mustela erminea;* Fig. 1). No ACE2 immunolabelling was observed in the lung of the least weasel (*Mustela nivalis*) or polecat (*Mustela putorius*).

Evaluation of tissues from a selection of phocids revealed the presence of a small amount of ACE2 immunolabelling in the bronchial epithelium of the grey seal (*Halichoerus grypus*) and hooded seal (*Cystophora cristata*), and bronchiolar epithelium of the grey seal and harbor seal (*Phoca vitulina*) (Fig. 2; Table 1). ACE2 immunolabelling was however not observed in the lung of the harp seal (*Pagophilus groenlandicus*).

Lung tissues from other miscellaneous wild mammals were also available and evaluated for ACE2 expression by immunostaining. ACE2 receptor expression was detected in the bronchiolar epithelium in the crested macaque (*Macaca nigra*) and in medium-sized arterioles in the red squirrel (*Sciurus vulgaris*). Expression of ACE2 was not detected in the lungs of the European hedgehog (*Erinaceus europaeus*) and the red-necked wallaby (*Notamacropus rufogriseus*).

In the small intestines, ACE2 immunolabelling on the enterocytes was ubiquitous in all mammalian taxa examined.

3.2. Detection of ACE2 receptor in the nasal turbinates and trachea from selected species

For a subset of species where tissue was available, we also examined ACE2 expression in the nasal turbinates and trachea (Table 2).

Among the artiodactyls, sparse ACE2 immunolabelling was detected in the nasal respiratory mucosa epithelium of the moose, alpaca (Fig. 1), and the nasal olfactory mucosa epithelium and submucosal glands of the moose and roe deer. Low levels of ACE2 were also detected in the tracheal epithelium of the moose and roe deer.

In the mustelids, ACE2 immunolabelling was intense and widespread across the nasal respiratory and olfactory mucosal epithelium of the European badger, stoat (Fig. 1) and pine marten (Fig. 3). Immunolabelling of ACE2 in the tracheal epithelium of the European badger (Fig. 1) was strong and widespread. ACE2 was not detected in the trachea of the stoat and pine marten.

In other miscellaneous wild mammals, ACE2 immunolabelling was detected in the nasal respiratory mucosa epithelium of the grey seal and Daubenton's bat (*Myotis daubentonii*) (Fig. 3), and in the olfactory mucosa epithelium of the pipistrelle bat (*Pipistrellus pipistrellus*). We also identified ACE2 immunolabelled positive cells in the nasal and tracheal epithelium in the Asiatic lion (*Panthera leo leo*) (Fig. 3). ACE2 was not detected in the nasal turbinate or trachea of the red squirrel.

4. Discussion

The anatomical distribution of ACE2 receptors has been demonstrated previously in several domesticated and some wild animals [6–8,36]. Here, we further evaluated the ACE2 receptor profile of the respiratory and intestinal tract from a larger group of wild mammals with a focus on artiodactyl, mustelid and phocid species. ACE2 expression was sparse in the upper and lower respiratory tract of several European and Asiatic deer species. In addition, low levels of ACE2 expression were also detected in the lung of the Bactrian camel and hippopotamus. In contrast, high levels of ACE2 were observed in the upper respiratory tract of the European badger and stoat, consistent with

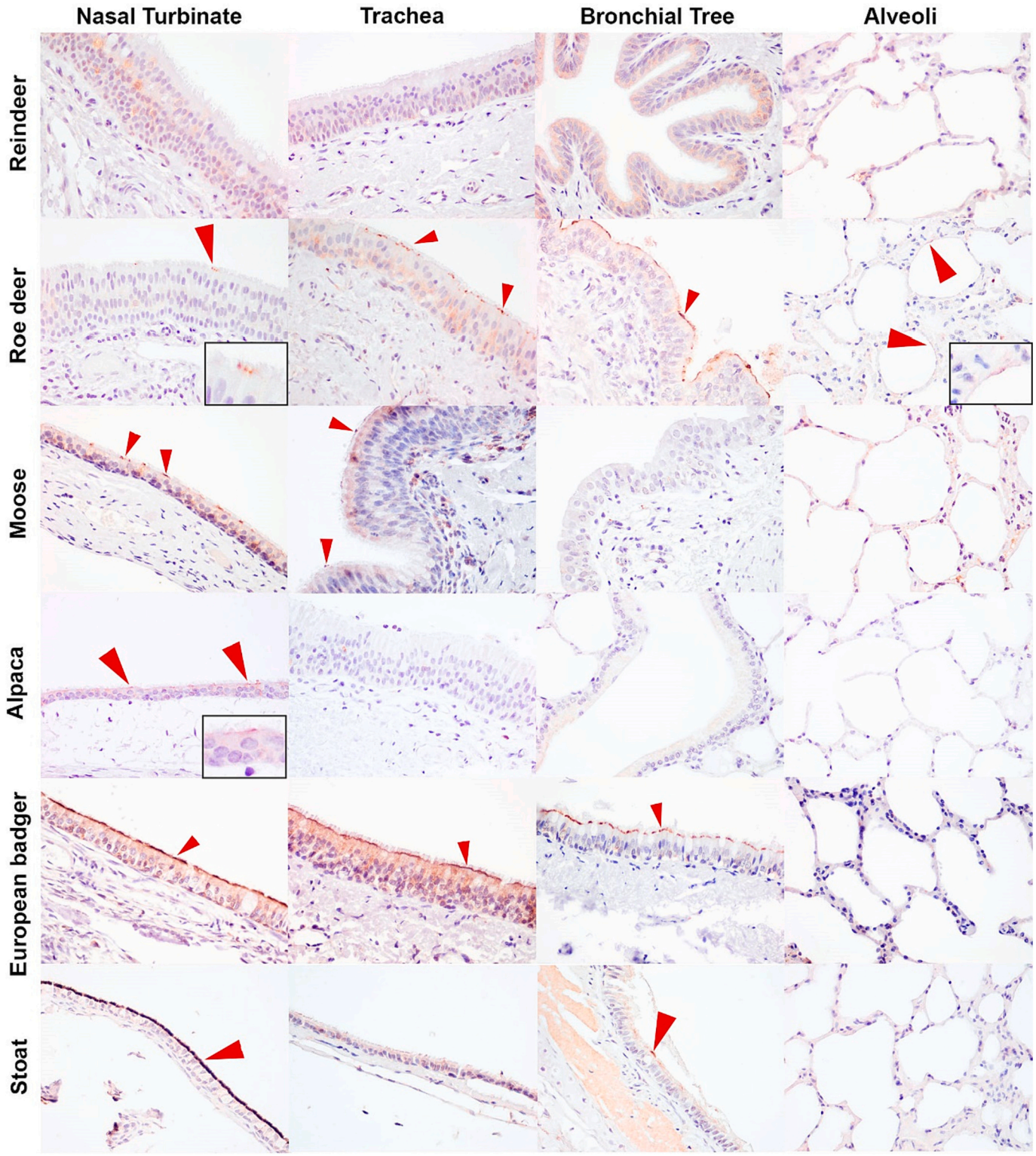

Fig. 1. Immunohistochemical labelling of ACE2 receptor expression in the respiratory tract of the artiodactyl and mustelid species. Positive ACE2 immunolabelling indicated with red arrow heads. ACE2 was absent in the upper and lower respiratory tract of the semi-domesticated reindeer (*Rangifer tarandus tarandus*). In roe deer (*Capreolus capreolus*), ACE2 immunolabelling was present in the olfactory epithelium (shown above) and submucosa glands (not shown). The immunolabelling was weak and represented by a discontinuous labelling pattern on the trachea and bronchiolar epithelial cells, and type I pneumocytes. In the moose (*Alces alces alces*), there was only weak and sparse ACE2 immunolabelling in the nasal respiratory (shown above) and olfactory (not shown) mucosa epithelium. ACE2 immunolabelling was rarely detected in the nasal respiratory mucosa epithelium in alpaca (*Vicugna pacos*; inset shows positive cells). The European badger (*Meles meles*) demonstrated strong and confluent ACE2 immunolabelling on the apical aspect of the nasal turbinate respiratory mucosa, trachea, and bronchiolar epithelium. Similarly, ACE2 expression was intense in the nasal turbinate respiratory (shown above) and olfactory mucosa (not shown) of the stoat (*Mustela erminea*) although bronchial immunolabelling was scattered. Images taken at 400× magnification. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

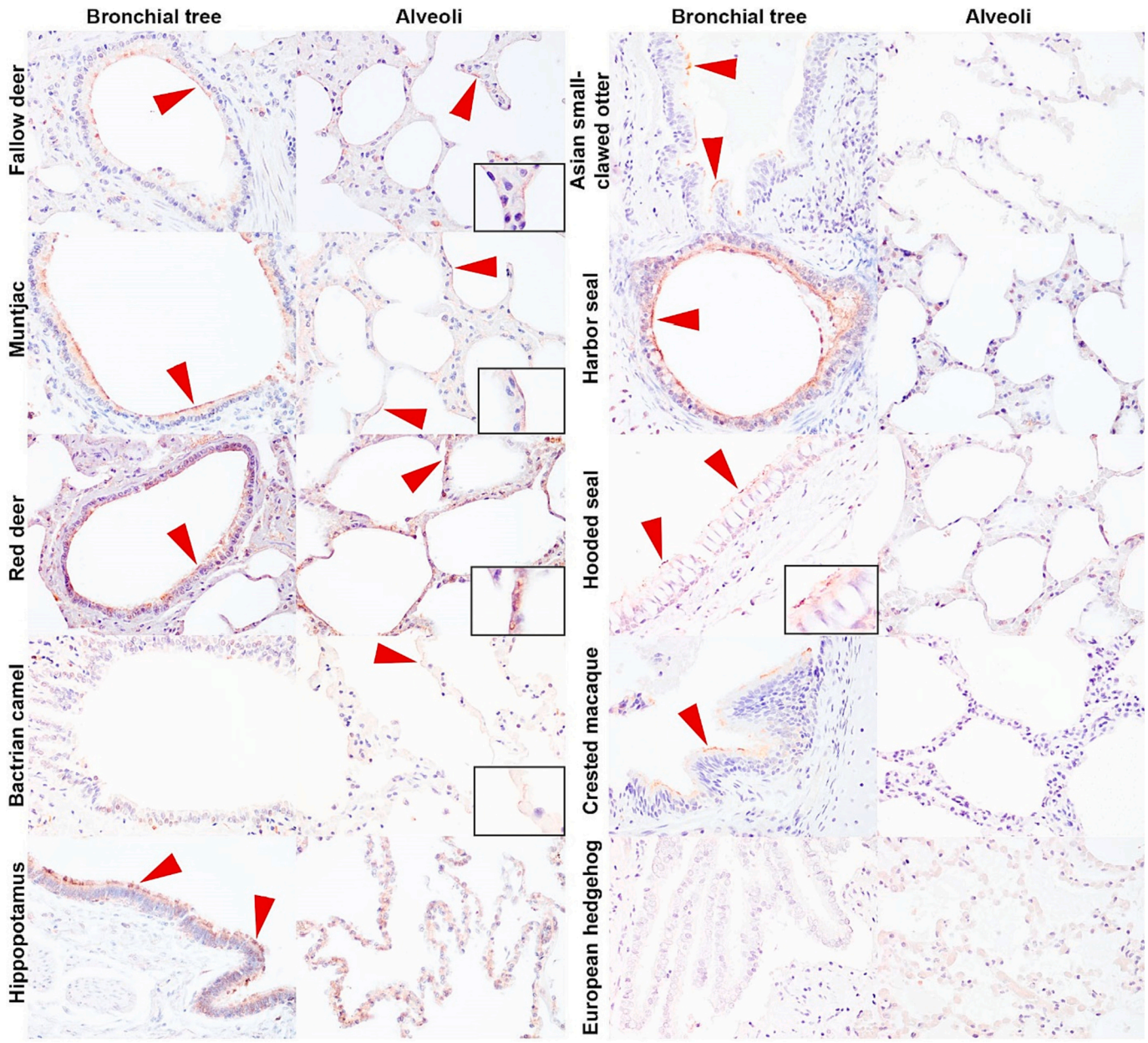

Fig. 2. Immunohistochemical characterisation of ACE2 receptor expression in the lung. Positive ACE2 immunolabelling indicated with red arrow heads. ACE2 was detected in the bronchiolar epithelium of fallow deer (*Dama dama*), muntjac (*Muntiacus reevesi*), red deer (*Cervus elaphus*), hippopotamus (*Hippopotamus amphibius*), Asian small-clawed otter (*Aonyx cinereus*), harbor seal (*Phoca vitulina*), and crested macaque (*Macaca nigra*), and in the bronchial epithelium of the hooded seal (*Cystophora cristata*). In the alveoli, ACE2 was present in type I pneumocytes of the fallow deer, muntjac, red deer and the Bactrian camel. Images taken at 400× magnification. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

previously reported observations for ferrets and mink [6,7]. ACE2 was not expressed in the lungs of other wild mammals including the European hedgehog and red-necked wallaby. Overall, the location and abundance of ACE2 receptor expression was shown to vary among closely related species.

European fallow deer, roe deer, and red deer are the most common cervid species in Europe, but other introduced species are also present on the European continent and in the UK, such as Chinese water deer, muntjac and sika deer [37,38]. Surveillance conducted in Germany, Austria and the UK to date has found no evidence of prior exposure to SARS-CoV-2 in deer species including fallow deer, roe deer, red deer, muntjac, Chinese water deer, sika deer and hybrid species [17,18]. Roe deer are phylogenetically closely related to WTD [39] and the ACE2 amino acid sequence between the two species is highly homologous [11,18]. In contrast to the predominant upper respiratory tract expression of ACE2 in the WTD [10], our findings indicate the presence of this receptor in both the upper and lower respiratory tract of roe deer. In addition, ACE2 receptor was also detected in the upper respiratory tract of the moose in the present study. Although *in vitro* infection of tracheal epithelial cells and precision cut lung slices from moose did not result in productive infection with SARS-CoV-2 [35], the presence of ACE2 receptor in the upper respiratory tract is an important finding as this anatomical location is more easily exposed to aerosolised virions than the lower respiratory tract. These findings suggest that roe deer and moose may be highly susceptible to infection with SARS-CoV-2. Often these cervids occupy peri-urban habitats, putting them in close proximity with humans and at potential risk of exposure to infection.

Among the other artiodactyls investigated, ACE2 receptor expression

Table 2
Immunohistochemical characterisation of ACE2 receptors in the nasal turbinate and trachea.

Family	Species	Country /Region Sampled	Free living (F), captive (C), domestic (D)	Sample size	Nasal turbinate	Trachea
Cervidae	Western roe deer (*Capreolus capreolus*)	Norway, Sweden	F	5	Olfactory epithelium and submucosa glands	Mucosa epithelium
	Moose (*Alces alces alces*)	Norway	F	3	Respiratory and olfactory epithelium and associated submucosal glands	Mucosa epithelium
	Norwegian semi-domesticated reindeer (*Rangifer tarandus tarandus*)	Norway	C	2	–	–
	Svalbard wild reindeer (*Rangifer tarandus platyrhynchus*)	Norway	F	4	–	–
Camelidae	Alpaca (*Vicugna pacos*)	UK	D	2	Respiratory nasal mucosa epithelium	–
Suidae	Wild boar (*Sus scrofa*)	UK	C	3	–	–
Mustelidae	European badger (*Meles meles*)	UK	F	2	Respiratory and olfactory epithelium	Mucosa epithelium, submucosa glands
	Stoat (*Mustela erminea*)	UK	F	5	Respiratory and olfactory epithelium	–
	European pine marten (*Martes martes*)	UK	F	1	Olfactory mucosal epithelium	–
Sciuridae	Eurasian red squirrel (*Sciurus vulgaris*)	UK	F	1	–	–
Felidae	Asiatic lion (*Panthera leo leo*)	UK	C	1	Respiratory and olfactory epithelium	Mucosa epithelium, submucosa glands
Phocidae	Grey seal (*Halichoerus grypus*)	UK	F	1	Respiratory epithelium	n/a
	Harbor seal (*Phoca vitulina*)	Norway	F	2	–	–
	Harp seal (*Pagophilus groenlandicus*)	Greenland	F	4	n/a	–
	Hooded seal (*Cystophora cristata*)	Greenland	F	4	n/a	–
Vespertilionidae	Common pipistrelle (*Pipistrellus pipistrellus*)	UK	F	2	Olfactory mucosal epithelium	n/a
	Daubenton's bat (*Myotis daubentonii*)	UK	F	1	Respiratory and olfactory epithelium	n/a

- negative; n/a, not available.

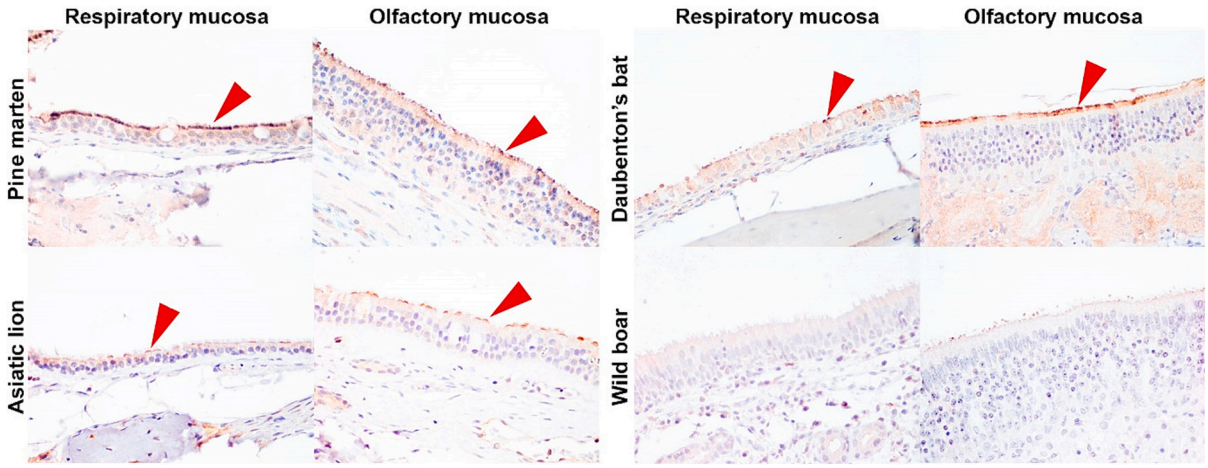

Fig. 3. Immunohistochemical labelling of ACE2 receptor expression in the nasal turbinates of selected species. Positive ACE2 immunolabelling indicated with red arrow heads. ACE2 was detected in the the respiratory (left column) and olfactory (right column) mucosa of the pine marten (*Martes martes*), Asiatic lion (*Panthera leo leo*) and Daubenton's bat (*Myotis daubentonii*). Images taken at 400× magnification. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

was detected in the bronchiolar epithelium of the hippopotamus and the Bactrian camel, and in the nasal turbinate of the alpaca. SARS-CoV-2 infection was detected in two captive hippopotamuses in Belgium following the onset of mucopurulent nasal discharge [40]. Although a variety of old and new world camelids are known to be susceptible to MERS-CoV infection (another *Betacoronavirus* related to SARS-CoVs [41–44], which utilises dipeptidyl peptidase-4 (DPP4) [42,45,46] rather than ACE2 as its receptor), host susceptibility to MERS-CoV cannot be used to predict susceptibility to SARS-CoVs. To date, SARS-CoV-2 infection has not been reported in camelids. While ACE2 was present in the nasal turbinate of the alpaca in our study, experimental inoculation of this species with SARS-CoV-2 did not result in productive infection [47]. Given the divergence of ACE2 receptor amino acid phylogeny between old and new world camelids [5], and preliminary evidence of ACE2 receptor expression in the respiratory tissues in this study, the susceptibility of the old and new world camelids to SARS-CoV-2 infection is unclear and warrants further investigation.

Following our previous report showing ACE2 receptor expression in

the lungs of the European badger [6], the present study has provided further evidence of ACE2 receptor expression throughout the respiratory tract of the European badger and stoat, both common mustelids in the UK. ACE2 was abundantly expressed throughout the upper and lower respiratory tract of the European badger, while in contrast ACE2 was only abundant in the upper respiratory tract of the stoat but rarely found within the bronchiolar epithelium. This resembles the distribution of ACE2 receptors in mink and ferrets with the presence of ACE2 in both the upper and lower respiratory tract of the former, and only in the upper respiratory tract of the latter. These differences may be related to virus tropism and differences in pathological presentation [6,7,27]. The lack of ACE2 receptor expression in the lung of the polecat, similar to the ferret [6], suggests a conserved expression profile in the lung of its domesticated descendant.

Although there have been no reports of SARS-CoV-2-related disease in other wild mustelid populations to date, recent surveillance in France has identified evidence of seroconversion in pine martens and European badgers [25]. There are also reports of other mustelids such as captive Asian small-clawed otters and a wild Eurasian otter testing positive for SARS-CoV-2 [26,48]; the susceptibility of the Asian small-clawed otters is likely related to the presence of ACE2 receptors in the lung as described in the present study. The current findings together with evidence from previous histological studies [6,7] demonstrate that the upper respiratory tract of various mustelids (ferret, mink, European badger, stoat, pine marten) appear to consistently express high levels of ACE2 receptor. The conserved and ubiquitous expression of ACE2 in the upper respiratory tract of a subset of mustelids is of concern considering that this is the first location that exposure to aerosolised virions may occur or may be in-contact with other infected individuals or fomites.

Mustelids such as American mink, the Chinese ferret badger (*Melogale moschata*), yellow-bellied weasel (*Mustela kathiah*) and Siberian weasel (*Mustela sibirica*) are known to be naturally susceptible to various coronavirus infections [49–51]. In production systems (e.g. fur farms) where competent hosts are kept at high stocking density, the incursion of coronavirus can lead to rapid spread within the population, as seen with SARS-CoV-2 in captive mink [20]. In some parts of the world, mustelids can also come into close contact with the public for example in live animal markets in Asia [52]. In addition to coronaviruses, some mustelids are competent hosts for other respiratory viruses, such as influenza A virus in naturally-infected Chinese ferret badgers and farmed mink [53,54], and in ferrets under experimental settings [55,56]. Collectively, these observations underline the need for further information about the potential involvement of mustelids in the emergence of respiratory pathogens.

Currently only limited information is available to assess the susceptibility of marine mammals to SARS-CoV-2 infection. Our study is the first to demonstrate expression of ACE2 receptors in the bronchial and bronchiolar epithelium of the grey, harbor and hooded seal. Although the ACE2 amino acid sequence of pinnipeds is highly similar to that of humans [29], *in silico* binding analysis between ACE2 orthologues and SARS-CoV-2 suggests that susceptibility of these species is likely to be low [5]. However, it is noteworthy that mink were predicted to have low susceptibility to SARS-CoV-2 based on molecular modelling which identified low amino acid homology and poor receptor binding affinity [5]. Clearly this proved not to be the case, likely as a consequence of the abundance of ACE2 receptors present in the upper and lower respiratory tract of mink [6,7]. This demonstrates the value of understanding the availability and distribution of ACE2 receptors when assessing the potential risks of SARS-CoV-2 exposure in wildlife.

Bats are a known natural reservoir for SARS-like viruses and harbor a diverse range of other coronaviruses, including some that utilise ACE2 receptor for cell entry [57–60]. Although the pandemic *Sarbecoviruses* (SARS-CoV and SARS-CoV-2) first emerged in Asia [60], other *Betacoronaviruses* including *Sarbecoviruses* have been detected in rhininolophus and vespertilionid bats in the Western Caucasus region of Russia and in Europe [61–64]. The results presented here and those reported previously [6] have demonstrated the expression of ACE2 receptors within the nasal mucosa epithelial cells of Daubenton's, pipistrelle, and serotine bats (all of which are insectivorous microchiroptera). The lower respiratory and intestinal tracts were not evaluated in this study as these tissues were autolysed. A previous report on DPP4 receptor mapping in bat tissues demonstrated differences in expression in the respiratory and intestinal tracts of insectivorous and frugivorous bats [65]. As observed with ACE2, the abundance and distribution of the receptors can vary widely among closely related species for instance in cervids and mustelids [6,7,10]. Given the paucity of information on ACE2 expression in bats and their potential importance as wild animal hosts of coronaviruses, further studies on both respiratory and intestinal tracts are necessary to provide a better understanding of the susceptibility of different species to ACE2-dependent virus infection.

In conclusion, our study showed that multiple cervid and mustelid species express ACE2 receptors in their respiratory and small intestinal tracts. We also present the first reports of ACE2 receptor distribution in phocids. Our results clearly identify substantial variation in the distribution and density of ACE2 receptor expression within taxonomic families, which suggests that extrapolation of results from closely related species (e.g. mink versus ferret, WTD versus roe deer) may be unreliable. With the continual evolution of SARS-CoV-2 driving changes in host tropism [66], and the potential for emergence of other ACE2 receptor-mediated SARS-related coronaviruses [57], further studies are warranted to characterise the anatomical location and the abundance of the cognate host receptor expression in other mammalian species. To better estimate the abundance or the level of receptor expression, transcript analysis such as by quantitative PCR [35] should be incorporated. The knowledge generated from ACE2 mapping will inform assessments of risk and targeted surveillance strategies for the potential incursion of ACE2 receptor-mediated viral infection in wild animal hosts that may have implications for human and domestic animal health.

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Ethical statement

No ethical approval was required as tissue blocks were derived from histology archives.

Author contribution statement

F.Z.X.L., J.G., S.A., L.E. performed the experiments. F.Z.X.L. conducted formal analysis. A.Nu., L.M., S.M.B. provided project leadership, financial, and laboratory resources. F.Z.X.L. wrote the original draft. All authors reviewed and edited the manuscript. R.C., R.J.D., K.M., S.S., I.H.N., B.L., C.B., A.Ne., P.H., C.M., L.P.F., E.W., U.G. provided carcasses or tissues.

Declaration of Competing Interest

The authors declare no conflict of interests.

Data availability

Data will be made available upon request.

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