SHORT REPORT
Peste des petits ruminants in Arabian wildlife

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SUMMARY
Recurrence of peste des petits ruminants (PPR) was diagnosed in the United Arabian Emirates in several wild ruminants confirmed by morphological, immunohistochemical, serological and molecular findings. Phylogenetic analysis revealed that the virus strain belongs to lineage IV, which is different to some previously isolated PPR strains from the Arabian Peninsula. This study shows that wild ruminants may play an important epidemiological role as virus source for domestic small ruminants.

Key words: Arabian Peninsula, peste des petits ruminants (PPR), wild ruminants.

Peste des petits ruminants (PPR) is a highly contagious disease caused by PPR virus (PPRV), a member of the genus *Morbillivirus* [1] that is widely distributed in sub-Saharan Africa, and various Asian countries including the Indian subcontinent [2, 3]. PPR has been diagnosed in several countries of the Arabian Peninsula during the past decades [4–6]. Molecular characterization revealed that virus strains isolated in the 1980s belonged to lineage III of PPRV [3, 7], whereas recently isolated strains were classified as lineage IV [7].

PPRV primarily infects goats and sheep [8], but wild ungulates including African Grey duiker (*Sylvicapra grimmia*), Dorcas gazelle (*Gazella dorcas*), Thomson’s gazelle (*Eudorcas thomsonii*), Nubian ibex (*Capra nubiana*), Laristan sheep (*Ovis gmelini laristanica*), and gemsbok (*Oryx gazella*) are also susceptible [4, 9, 10]. The role of wildlife in the epizootiology of PPR is still unknown. In this study, morphological, immunohistochemical, and molecular findings of two recent outbreaks of PPR in wild small ruminants in the United Arab Emirates (UAE) are described.

In winter 2005/2006, a disease outbreak with diarrhoea and more than 100 fatalities in a private collection of different wild small ruminants kept under semi-free-range conditions in the UAE was observed. An additional disease outbreak occurred in another private collection in the UAE in winter 2008/2009 with a mortality rate of almost 100%. In total, four antelopes (three bushbucks, *Tragelaphus scriptus*; one impala, *Aepyceros melampus*), 14 gazelles of four different species (four Rheem gazelles, *Gazella subgutturosa marica*; seven Arabian mountain gazelles, *Gazella gazella cora*; one springbuck, *Antidorcas marsupialis*; two Arabian gazelles, *Gazella gazella*), six Nubian ibex (*Capra nubiana*), eight Barbary sheep (*Ammotragus lervia*), and one Afghan Markhor goat (*Capra falconeri*) were submitted for necropsy.

Necropsy findings, almost similar in all animals, included catarrhal to haemorrhagic colitis, occasionally also abomaso-enteritis, with linear haemorrhages...

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in the colonic mucosa (so-called zebra stripes), splenomegaly, pulmonary congestion with occasional suppurative pneumonia, and oedema of body and organ lymph nodes. Lesions of the oral mucosa were not observed.

Histological examination revealed necrotizing to haemorrhagic enteritis/colitis with lymphoid necrosis and depletion of the gut-associated lymphoid tissue. Lymphoid depletion and necrosis occurred also in the spleen and lymph nodes. The lungs showed subacute broncho-interstitial pneumonia with hyperplasia of type II pneumocytes and bronchiolar epithelial cells and presence of syncytial cells. Suppurative to fibrinopurulent bronchopneumonia was seen occasionally. In the liver, multifocal hepatocellular coagulation necroses with infiltration of macrophages were present. Eosinophilic cytoplasmic and nuclear inclusion bodies were found in gastrointestinal epithelial cells, macrophages/reticular cells of lymphoid tissues, bronchial and bronchiolar epithelial cells, syncytial cells and biliary epithelial cells (Fig. 1).

Immunohistochemistry was performed on selected tissues from animals of both outbreaks as described previously [11]. Morbillivirus antigen was found in the cytoplasm and nucleus of intestinal epithelial cells, bronchial and bronchiolar epithelial cells, syncytial cells, bile duct epithelia, hepatocytes, and cells of intestinal lymphoid follicles.

Virus isolation was attempted on different cell monolayers including Vero, MDBK, and lamb testis cells with samples of spleen, lymph node, lung, and brain from selected animals of both outbreaks. The flasks were incubated for 7–14 days and passaged three times without successful isolation of a virus.

Serum from animals of the second outbreak was examined for antibodies using specific Rinderpest virus (RPV) and PPRV monoclonal antibody-based competitive enzyme-linked immunosorbent assays (cELISA; Biological Diagnostic, UK). Seven samples were positive in the PPRV-cELISA, and all were negative for RPV.

For molecular analysis samples from small and large bowel as well as intestinal lymph nodes were used from two Barbary sheep and one Nubian ibex of the second outbreak. The RNeasy Maxi kit (Qiagen, Germany) was applied for RNA isolation, followed by a DNase treatment according to the manufacturer’s instructions. Subsequently, RT–PCR was performed using random primers (Promega, Germany) and the Omniscript RT–PCR kit (Qiagen). For identification of morbillivirus RNA, a set of universal morbillivirus primers (sense primer: 5’-atgtttatgatcacagcggt-3'; antisense primer: 5’-tttggttgcaccacttgtc) was used [12]. For differentiation of PPRV and RPV, specific primer pairs located on the N protein gene sequence of each virus type (PPRV sense: 5’-gtctcgaaatcgcctcacagact-3'; PPRV antisense: 5-ctctctctgctctcagact-3; RPV sense: 5’-caaggggtattgatgacagca-3'; RPV antisense: 5’-atctttgttttgtatgtcge-3') were used [7]. The identity of all obtained PCR products was analysed by sequencing (Agowa GmbH, Germany). A 296-bp amplicon of the N gene (GenBank accession no. FJ795511) was detected in all tissues investigated. Phylogenetic analysis using the N protein gene sequences of 31 PPRV strains of different geographic origins showed that the novel PPRV Dubai strain belongs to lineage IV of PPRV. It is closely related to a recently isolated Chinese PPR strain and relatively distant to strains previously identified in Arabian wildlife (Fig. 2).

Clinical information of the described outbreaks was scarce, but the reported diarrhoea and high mortality are similar to PPR outbreaks in naive small domestic ruminants [13] and gazelles [4]. There is very limited information on species susceptibility and the occurrence of PPR in wild ungulates. The susceptibility for PPR varies in wild ruminant species [4, 14], but the described outbreaks of PPR in the UAE extend the spectrum of susceptible species by bushbuck, impala, Rheem gazelle, Arabian mountain gazelle,
springbuck, Arabian gazelle, Barbary sheep, and Afghan Markhor goat.

The morphological findings in the investigated wild ruminants share some features of those in sheep and goats with PPR [8]. Pneumonia, necrosis and depletion of various lymphatic tissues belong to the spectrum of typical lesions in PPR of small domestic ruminants [8]. Erosive mucosal lesions in the upper digestive and respiratory tract, which are characteristic for PPR in sheep and goats [8], were not found. Detailed morphological descriptions of PPR in wild ruminants are missing, but in two sacrificed gazelles small erosions were observed on the tongue [4].

Immunohistochemistry demonstrated morbillivirus antigen in various epithelial tissues and lymphatic organs. The viral antigen distribution in wild ruminants is similar to that described for sheep and goats [15]. Immunohistochemistry is an alternative and rapid method for diagnosing a morbillivirus infection, especially because cultural virus isolation using various types of cells failed in the described outbreaks. Serological testing on PPR-specific antibodies confirmed the diagnosis.

Molecular analysis identified a strain (PPRV/Dubai; GenBank accession no. FJ795511) belonging to lineage IV of PPRV based on phylogenetic analysis of the N protein [7]. PPRV/Dubai is closely related to a recently characterized strain from China, but is distant to lineage IV strains originating from gazelles isolated 1999 and 2002 in Saudi Arabia (GenBank accession No. DQ840195, DQ840197 and DQ840196), and is not related to lineage III strains isolated from a Dorcas gazelle in the UAE in 1986 [9] (GenBank accession no. DQ840169) and sheep and goats [10].

Fig. 2. Unrooted neighbour-joining phylogenetic tree constructed by using 296 nt from the gene coding for the PPRV nucleocapsid (N) protein. Alignments were calculated with ClustalX version 2.0.11 (ftp://ftp.ebi.ac.uk/pub/software/clustalw2). Bootstrapping (values indicated in %) was performed with 1000 replicates using MEGA 4.1 software (www.megasoftware.net/mega.html). The new isolate PPRV/Dubai 2009 (GenBank accession no. FJ795511) from this study is shown in boldface. It was compared to 31 other peste des petits ruminant virus (PPRV) strains isolated from different outbreaks. Scale bar shows nucleotide substitutions per site. II–IV represent lineages I–IV of PPRV.
goats in Oman in 1983 [6] (GenBank accession no. DQ840168). The origin of the novel strain PPRV/Dubai remains unknown. Import of infected domestic or wild small ruminants from Asia into the UAE or other countries of the Arabian Peninsula has to be considered as a possible source of infection. However, cross-border introduction from neighbouring countries, where other strains of the lineage IV are circulating [5], seems less likely. Susceptibility of wild ruminants for PPRV is epidemiologically important, because domestic and wild ruminants are mingling together allowing inter-species transmission. PPRV obviously circulates uncontrolled among wildlife representing a potential virus source for domestic species.

DECLARATION OF INTEREST
None.

REFERENCES