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Bat Coronaviruses circulating in Danish bats

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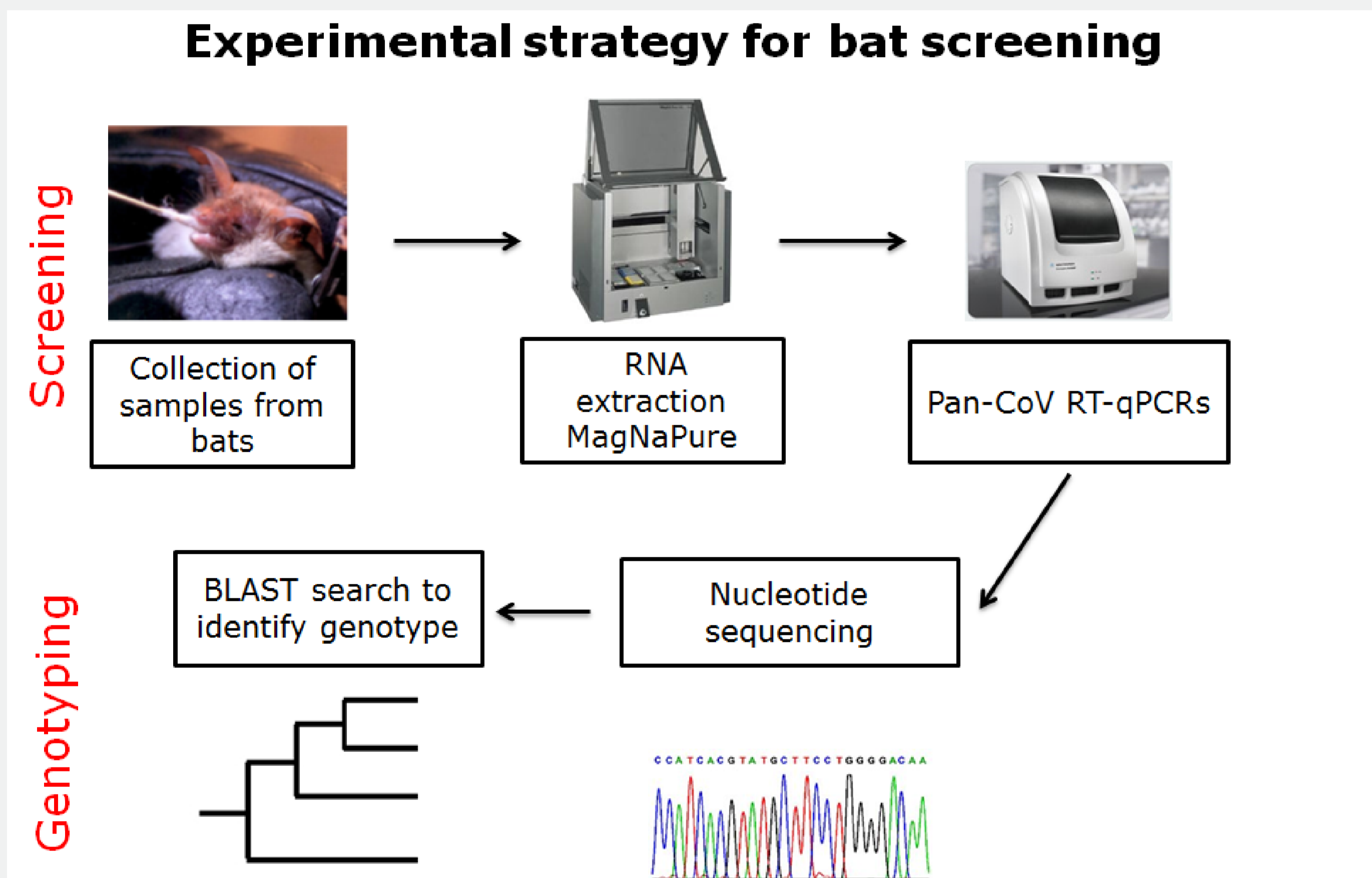
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OBJECTIVE

Coronaviruses (CoVs) are a diverse group of large positive-stranded RNA viruses. Several CoVs are known to cause severe diseases in animals (e.g. PED and TGE) and humans (e.g. SARS and MERS). Bat populations worldwide are considered to be natural reservoirs for many diverse CoVs. However, incomplete information exists about bat species hosting specific CoVs and also the nature of bat CoVs circulating in Europe.

In this study, we investigated healthy bat populations in Denmark for the presence of CoVs.



METHODS

Fecal samples were collected during the autumn of 2013 and 2014 from 10 out of 17 known Danish species of bats that were caught alive and released after sampling.

The samples were screened for the presence of CoV RNA using pan-CoV RT-qPCRs targeting conserved regions of the ORF1ab that encodes the viral RNA polymerase.

Amplicons that were generated were sequenced to confirm the presence of CoV and to determine the type and diversity of CoVs among Danish bat species.

RESULTS

In total, 21 fecal samples from 4 different bat species were found positive by two independent pan-CoV RT-qPCRs. The majority of positives were detected in *M. daubentonii* (n=15), but CoV RNA was also found in *M. nattereri* (n=1), *P. pygmaeus* (n=2) and *E. serotinus* (n=1).

The nucleotide sequencing revealed distinct CoV sequences within each of the four bat species suggesting that specific CoVs may be confined to single host species.

BLAST analysis revealed up to 85% nucleotide identities compared to published alpha-CoVs...

Species	No. Sampled (No. Positive)	Date of Collection
<i>Myotis daubentonii</i>	4 (0)	Sept 2013
	40 (7)	Oct 2013
	53 (8)	Oct 2014
<i>Myotis dasycneme</i>	2 (0)	Oct 2013
	6 (0)	Oct 2014
<i>Myotis nattereri</i>	7 (0)	Aug-Sept 2013
	1 (0)	Sept 2013
	2 (1)	Oct 2014
<i>Myotis bechsteinii</i>	4 (0)	Aug 2013
<i>Myotis mystacinus</i>	1 (0)	Aug 2013
<i>Myotis brandtii</i>	2 (0)	Aug 2013
<i>Nyctalus noctula</i>	4 (0)	Aug 2013
<i>Pipistrellus pygmaeus</i>	6 (1)	Aug 2013
	9 (1)	Jun 2014
<i>Plecotus auritus</i>	1 (0)	Aug 2013
<i>Eptesicus serotinus</i>	3 (3)	Jun 2014
Total	145 (21)	

Alignment of CoV nucleotide sequences obtained from bats. The image shows a multiple sequence alignment of CoV nucleotide sequences from various bat species and years (2013 and 2014). The sequences are aligned in columns, with positions 20, 40, 60, 80, 100, and 120 marked. The sequences are color-coded by nucleotide: C (blue), A (green), T (red), G (black). The alignment shows high similarity between sequences from the same species and year, and some differences between species and years.

Alignment of CoV nucleotide sequences obtained from bats.

CONCLUSIONS

This study presents the first record of CoV in Danish bats.

Our results show, that several distinct alpha-CoVs are present in the Danish bat population and they may have different host species..