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Short communication

Detection of Neospora caninum DNA by polymerase chain reaction in bats from Southern China

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ABSTRACT

Neospora caninum is an intracellular protozoan that infects many domestic and wild animals. Domestic dogs and other canids function as definitive hosts, while other mammals serve as natural intermediate hosts. In the present study, the brain tissues of bats collected in Yunnan Province, Southern China were tested by \textit{N. caninum} specific-nested PCR, targeting the \textit{Nc-5} gene and the internal transcribed spacer 1 (ITS1) region of the ribosomal DNA to determine whether bats could be infected with \textit{N. caninum}. \textit{N. caninum} DNA was detected in 1.8% (4/227) of bats, i.e., 1.7% (1/60) in \textit{Roussetus leschenaultia}, 1.7% (1/58) in \textit{Hipposideros pomona}, 2.9% (2/69) in \textit{Rhinolophus pusillus}, and none (0/40) in \textit{Myotis daubentonii}. The findings of the present study are only the first indication that bats could serve as an intermediate host, and further studies are necessary to confirm whether bats are involved in the transmission of \textit{N. caninum} infections.
the ribosomal DNA in *N. caninum* using the primers NN1 and NN2 as external primers, and NP1 and NP2 as internal primers [12]. Positive and negative controls were included in each test. The genomic DNA of the *N. caninum* NC-1 strain was used as positive control, and ultrapure water was used as negative control. The PCR products were electrophoresed in a 1.5% agarose gel and stained with an ethidium bromide solution (1 µg/ml). The sample was considered positive if it was tested positive by the two previously mentioned methods.

The PCR products of all positive samples were purified using the TaKaRa MiniBEST Agarose Gel DNA Extraction Kit Ver.4.0 (Takara Biomedical Technology Co., Ltd., Beijing, China) according to the manufacturer’s protocol. The PCR product was cloned into vector pMD18-T (Takara) and sequenced using the primers M13-47 and RV-M according to the manufacturer’s protocols. The PCR product was cloned into vector pMD18-T (Takara) and sequenced using the primers M13-47 and RV-M in an ABI 3730 sequencer.

The sequences were compared with the GenBank entries by Blast [6] and the GenBank accession numbers for ITS1 are MF802344, and for ITS2 are MF802334, MF802335, and for ITS1 are MF802334, MF802335, and MF802336.

Many wild animals are considered intermediate hosts of *N. caninum*. For example, antibodies to *N. caninum* have been detected 61.5% in eland, 58.5% in zebra, 19.2% in gazelle, 33.3% in warthog, 50% in African buffalo, 30% in lion, 20% in cheetah, and 33.3% in spotted hyena by an agglutination test in Kenya [16], and 8.4% in white-tailed deer by ELISA in Northern Mexico [17]. *N. caninum* DNA has been found 6% in magpie in Spain by PCR [18], and 3.7% in house sparrows in Iran [19]. These results show that these wild animals may be infected or contact with the parasite.

We detected PCR positive findings for *N. caninum* DNA in the brain tissue of one fruit bat and three insectivorous bats in this study. Previous studies have indicated that insectivores and insectivorous bat may be a risk factor for domestic animals to get infected with *Toxoplasma gondii* and *N. caninum* [20]. Recently, a study conducted in Brazil showed a negative result of *N. caninum* infection in 97 bats, including 12 fruit bats, 49 insectivorous bats, and 36 vampire bats. These results may be related to the contamination of oocysts in the environment, the susceptibility of bats to *N. caninum*, and the sensitivity of detection methods. We used nested-PCR methods targeting *Nc*-5 and ITS1 genes to detect *N. caninum* DNA in bats, which may be helpful to increase the positive detection rate of the parasite. Unfortunately, the bat tissue had been used for the detection of viruses and parasites and was no longer suitable for histopathological analysis to confirm the molecular detection of *N. caninum* [21, 22].

In conclusion, this is the first report on the molecular detection of *N. caninum* in bats from Yunnan Province, Southern China. Further studies are needed to confirm these molecular findings and whether bats are involved in the life cycle and transmission of *N. caninum*.

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The following are the supplementary data related to this article.

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### Table 1

| Target gene    | No. of positive samples/total (%) |
|----------------|----------------------------------|
| *Roussetus leschenaultia*<sup>a</sup> | 1/60 (1.7)                         |
| *Hipposideros pomona*<sup>b</sup>   | 2/58 (3.5)                         |
| *Rhinolophus pusillus*<sup>c</sup>  | 2/69 (2.9)                         |
| Myotis daubentonii<sup>d</sup>      | 4/69 (5.8)                         |
| Total           | 5/227 (2.2)                        |

<sup>a</sup> Fruit bat in the Pteropodidae family; GenBank accession number for *Nc*-5 is MF802336, and for ITS1 is MF802339.

<sup>b</sup> Insectivorous bat in the Hipposideridae family; GenBank accession numbers for *Nc*-5 are MF802334-MF802335, and for ITS1 is MF802344.

<sup>c</sup> Insectivorous bat in the Rhinolophidae family; GenBank accession numbers for *Nc*-5 are MF802337-MF802338, and for ITS1 are MF802340-MF802343.

<sup>d</sup> Insectivorous bat.
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