

Short Communication

A Survey of *Neospora caninum* infection in sparrows (*Passer domesticus*) in Khuzestan Province, Iran

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ABSTRACT

The present study reports the presence of *N. caninum* DNA by PCR in brain tissues of house sparrows. The results showed that 6 (2.8%) of brain samples were positive for *N. caninum*. This is the first report for detection of *N. caninum* in sparrows, in Khuzestan Province. The results suggest that the meat of infected sparrows can be the source for dogs' infection.

Keywords: *Neospora caninum*; Sparrows; PCR; Khuzestan Province

INTRODUCTION

Neospora caninum is an apicomplexan parasite that has been extensively studied in the last two decades, due to its importance as a veterinary pathogen (Shivaprasad *et al* 1989). The presence of birds on dairy farms has been associated with outbreaks of abortion and proposed as a risk factor for *N. caninum* infection (Ould-Amrouche *et al* 1999, Bartels *et al* 1999, Otranto *et al* 2003). It has been shown that chickens may be a permissive intermediate host for *N. caninum* since parasite DNA has been detected in tissues from birds kept outdoors (Costa *et al* 2008). Furthermore, investigation of infection in birds is a useful way to access environmental contamination with oocysts, since different avian populations feed directly on the ground and are continuously exposed to oocyst ingestion (Dubey *et al* 2002, Waap *et al* 2008). The sparrow (*Passer domesticus*) is a cosmopolitan bird

that belongs to the order Passeriformes. The house sparrow is strongly associated with human habitations, and can live in urban or rural settings (Hatch & Westneat 2007). They were introduced in many parts of the world in the 19th and 20th centuries to control insect populations and for other purposes, and became the most abundant synantropic wild bird (Summers-Smith 1988). Since, the presence of birds is significantly correlated to higher *N. caninum* infection in dairy farms; the aim of this study was to evaluate the presence of *N. caninum* infection in sparrows in Khuzestan province.

MATERIALS AND METHODS

Whole brain was obtained from 210 dead sparrows slaughtered for meat at avian markets in different parts of Ahvaz city- the capital of Khuzestan province. DNA was extracted using a genomic DNA purification kit (SinaClon Bioscience, Iran). For detection of *N.*

caninum, primers targeting Nc5 gene were selected from the literature (Kang *et al* 2009). Primers (Bioneer, South Korea) used in the reaction were the forward primer Np 21 with the sequence 5'-CCCAGTGCGTCCAATCCTGTAAC-3' and the reverse primer Np 6 with the sequence 5'-CTCGCCAGTCAACCTACGTCTTCT-3', yielding a 338 bp product. PCR reactions included a negative control, consisting of the reaction mix and 2 µl of DNase/RNase-free water instead of DNA and a positive control consisting of DNA sample from the tachyzoites of *N. caninum* NC- isolate. All PCR were performed in a 25 µl reaction containing 12.5 µl Taq DNA polymerase master mix Red (Amplicon, Denmark), 1 µM primers and 50 ng DNA templates. PCR cycling included an initial denaturation at 94°C for 4 min, followed by 30 cycles of denaturation at 94°C for 50s, annealing at 56°C for 50s, extension at 72°C for 60s. This was followed by a final extension at 72°C for 5 min. PCR reactions included a negative control, consisting of the reaction mix and 2 µl of DNase/RNase-free water instead of DNA and a positive control consisting of DNA sample from the *N. caninum* tachyzoites (NC- 1 isolate). PCR products were electrophoresed in 1.5% agarose (SinaClon Bioscience, Iran) in Tris-acetate-EDTA (TAE) buffer, stained with Green Safe stain (SinaClon Bioscience, Iran) and visualized under ultraviolet light. Positive samples showed a band of approximately 338 bp. Amplified fragments corresponding to the size predicted for *N. caninum* were purified using a PCR purification kit (Fermentase, Lithuania) and were sent for sequencing (Sequence Laboratories, Goettingen, Germany). The obtained sequences were compared with those of *N. caninum*, already registered in the GenBank™ database. All the comparison and alignments were conducted using the nBLAST system (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>).

RESULTS AND DISCUSSION

In the present study samples were considered positive if they produced a band approximately 338 bp,

similarly to that of the positive control. The overall prevalence of *N. caninum* in sparrows was 2.8% (6/210). Positive samples were sequenced in order to verify the positive result. All sequenced samples were found by BLAST analysis to be closest to the *N. caninum* Nc 5 gene in GenBank with a similarity of ≥98% (GenBank™ accession nos. EF 202080.1, EF 202082.1, EU 073599.1 and KF 649846.1).

There are reports in the literature suggesting that the presence of birds in cattle-grazing farms could be associated with the increase of seroprevalence and abortion storms related to *N. caninum* infection (Bartels *et al* 1999, Otranto *et al* 2003). In *T. gondii* epidemiological chain, birds are considered important parasite reservoir, since those animals are frequently preyed upon by felids, its definitive hosts. Same pattern of events may be observed at the relationship between dogs and birds, which may lead to speculations if birds may also perform the role of *N. caninum* reservoirs in nature. The first identification of naturally infected birds with *N. caninum* has been recently reported in free-ranging chickens (Costa *et al* 2008). Molina-Lopez *et al.* (2012) reported a prevalence of antibodies in 35.8% of 67 common ravens. The results of Mineo *et al.* (2009) study showed that pigeons are a suitable model for *N. caninum* infection. Their results demonstrated that *N. caninum* disseminated through various tissues of pigeons experimentally infected with *N. caninum* and induced parasite specific IgG seroconversion. For the first time Gondim *et al.* (2010) introduced sparrows as the intermediate host and *Neospora* DNA was detected in 7.5% (3/40) of their samples. The present study is the first report of molecular detection of *Neospora* parasite from birds in Khuzestan province. The present investigation indicated that 2.8% of sparrows were infected with *N. caninum*. This could be related to the sparrow's nutrition. Sparrows commonly feed directly on the ground and are probably exposed to *N. caninum* after ingestion of the parasite oocysts from the soil. The results we obtained indicated soil contamination due to *N. caninum* oocysts because sparrows feed from the

ground, and suggested that the meat from the poultry might be an important source for dog infection by *N. caninum*. In conclusion, DNA of *N. caninum* observed in 2.8% of house sparrows from Iran and suggested that the meat from the sparrows might be an important source for dog infection by *N. caninum*.

Ethics

I hereby declare all ethical standards have been respected in preparation of the article.

Conflict of Interest

Hereby, I declare "no conflict of interest exists" regarding submitted article.

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