

Original papers

Molecular detection of *Neospora caninum* infection in ovine aborted foetuses in the Mashhad area, Iran

Gholamreza Razmi¹, Zahra Naseri²

¹Department of Pathobiology, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Azadi Square, P.O. Box 91775-1793, Mashhad, Iran

²Center of Excellence in Ruminant Abortion and Neonatal Mortality, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Azadi Square, P.O. Box 91775-1793, Mashhad, Iran

Corresponding Author: Gholamreza Razmi; e-mail: razmi@um.ac.ir

ABSTRACT. *N. caninum* could cause abortion in small ruminants. The aim of the study was to detect *N. caninum* infection in ovine aborted foetuses in the Mashhad area by PCR examination. During the period 2009 to 2013, 71 ovine aborted foetuses were collected and their brain samples examined by PCR. Of the 71 brains of the aborted foetuses, *N. caninum* DNA was detected in seven (9.8%) samples. In conclusion, it seems that *N. caninum* may act as a causative agent of abortion in sheep in the Mashhad area.

Key words: *Neospora caninum*, abortion, sheep, PCR

Introduction

Neospora caninum is an important causative agent of abortion in dairy cattle. It has a similar biology to *T. gondii* and can infect a wide range of animal species including dogs, cattle, horses, deer and foxes, as well as sheep and goats [1,2]. *N. caninum* has been sporadically reported as a cause of reproductive disorders in sheep [3–9]. Serological studies based on antibodies against *Neospora* indicate the prevalence of *N. caninum* infection to be 3% in sheep in Argentina [10], 8% to 31% in sheep in Brazil [11,12], 10.3% in sheep in China [9], 27.7% in sheep in Pakistan [13], 0.6% in sheep in New Zealand [14], 16.8% in sheep in Greece [6], 3.7% in sheep in Slovakia [15], 12% in sheep in the Czech Republic [16] and 10.1% in sheep in Spain [17]. In Iran, the seroprevalence of *N. caninum* infection in sheep was found to range from 1.1 to 5.7% depending on the province [18–20]; it was also detected in the brains and hearts of sheep [21] and the brains of aborted sheep foetuses by PCR [18,22]. *N. caninum* infection has been reported to be associated with abortion in dairy cattle in the Mashhad area [23,24]. As large and small ruminants commonly share the sources of

food and water, *N. caninum* may be have a role in inducing abortion of sheep in this area.

The aim of the study was to detect the presence of *N. caninum* infection in aborted ovine foetuses in the Mashhad area by PCR.

Materials and Methods

Sample collection. From August 2009 to August 2013, seventy-one aborted ovine foetuses at last two months of gestation were collected. At necropsy, the skull was opened under aseptic condition and the brain was sampled for PCR.

DNA extraction and PCR. The genomic DNA of brain samples were extracted using an MBST Genomic DNA kit (Institute of Molecular and Biological transmission systems, Tehran, Iran) as per the manufacturer's recommendations. For the detection of *N. caninum* DNA, PCR was performed as previously described by Müller et al. [25] with *N. caninum*-specific primers Np21plus (5' CCC AGT GCG TCC AAT CCT GTA AC 3') and Np6plus (5' CTC GCC AGT CAA CCT ACG TCT TCT 3'). Five µl of the PCR product was subjected to electrophoresis in a 1.5% agarose gel with TAE buffer, submerged in an ethidium bromide tank for

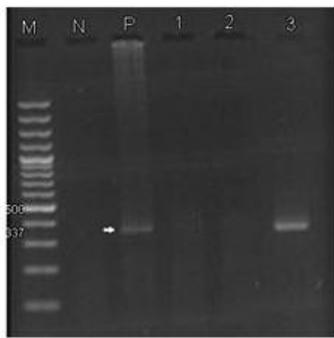


Fig. 1. PCR amplification products of *N. caninum* in brain samples

Lanes: M - molecular weight marker (between 1000 and 100bp); P - positive control (Nc-1 strain of *N. caninum*); N - negative control; 1,2 – negative samples; 3-positive sample

15 min. Distinct bands of *N. caninum* (337 bp) were visualized by UV illumination. A 100 bp molecular marker was used for size estimation (Fermentas®, Lithuania). An Nc-1 strain of *N. caninum* was used as the positive control and distilled water as a negative control for each PCR amplification.

Results

The age of all collected aborted foetuses were above 120 days. The DNA was successfully extracted from the brain samples. The primers amplified a 337bp fragment (Fig. 1). *N. caninum* DNA was detected in seven (9.8%) of the foetal brain samples.

Discussion

The main agent of protozoan abortion in sheep is classically related with *T. gondii*. As the morphology of *T. gondii* and *N. caninum* and the lesions they induce in abortion cases are very similar, ovine abortion due to *N. caninum* infection may be underdiagnosed [26]. With the development of molecular methods and the possibility of differentiation between the *T. gondii* and *N. caninum*, it has been confirmed that *N. caninum* could act as a causative agent of abortion in sheep flocks in the field [8,27].

In the present study, *N. caninum* was detected in the brains of seven of the 71 examined aborted foetuses. In Iran, *N. caninum* infection has been detected in 1 to 8.5% of ovine aborted foetuses by various PCR assays [18,22]. In other countries, *N. caninum* PCR positivity was found in 25% of ovine aborted foetuses in a flock in Switzerland [5], in

4.7% of ovine aborted foetuses in Spain [27], and in 2% of ovine aborted foetuses in Italy [28]. Recently, *N. caninum* DNA was detected in the brains of 13 of 14 aborted foetuses from seropositive sheep in Spain [8]. The detection of *N. caninum* DNA in aborted ovine foetuses suggests that *N. caninum* may be a causative agent of abortion in flocks of sheep. Further investigation using foetal histopathology combined with PCR is needed to confirm this hypothesis in ovine aborted foetuses in Iran.

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