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Short Communication

Vertical transmission of *Theileria lestoquardi* in sheep

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ABSTRACT

This is the first report of an outbreak of *Theileria lestoquardi* abortion and stillbirth in a mob of 450 ewes in July 2012, during which, approximately 35 late-term ewes lost their fetuses over a 5-day period. A dead ewe and her aborted fetus were transported to the Ahvaz Veterinary Hospital for the diagnostic evaluation. The microbial cultures from the ewe vaginal discharges and fetal abomasal contents and the liver were negative. The blood films of the ewe and her fetus contained *Theileria* piroplasms and the impression smears from ewe liver and fetal spleen were positive for *Theileria* Koch blue bodies. The DNA was extracted from the liver and spleen of ewe and her fetus, respectively, and analyzed by polymerase chain reaction (PCR) using specific primers derived from the nucleotide sequences of 18S ribosomal DNA (rDNA) gene of *T. lestoquardi*. A single fragment of 428-bp fragment was amplified. The PCR product was directly sequenced and the alignment of the sequence with similar sequences in GenBank® showed 100% identities with 18S rDNA gene of *T. lestoquardi*. The present study is the first report of the *T. lestoquardi* vertical transmission that could be related to the abortion.

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1. Introduction

Generally, economic importance of malignant theileriosis in sheep is related to mortality. The disease is active in the tropical parts, the south and southwest, of Iran (Hashemi-Fesharaki, 1997; Zaeemi et al., 2011). The ticks of *Hyalomma* sp. are responsible for the transmission of the disease in the region (Hooshmand Rad and Hawa, 1973).

Vertical transmission of theileriosis has been reported in horse (Allsopp et al., 2007) and cows (Baek et al., 2003). Intrauterine transmission of *Theileria equi* has been shown in foal (Allsopp et al., 2007). Infection of horse fetus with *T. equi* may lead to abortion, stillbirth, or the birth of live foal with neonatal piroplasmosis (Allsopp et al., 2007; Phipps and Otter, 2004). Vertical transmission of *Theileria sergenti* in cows was confirmed by polymerase chain reaction (PCR) (Baek et al., 2003).

The current study is the first report of the concurrent isolation of *T. lestoquardi* from a ewe and her aborted fetus.

2. Materials and method

In July 2012, a mob of 450 ewes, in southwest of Iran, was examined for approximately 35 late-gestation abortions and 17 ewes' mortality for a period of 5 days. A case of recently aborted fetus with the respective ewe was transported to the Ahvaz Veterinary Hospital for more diagnostic evaluations. Depression and fever (41.8 °C) were

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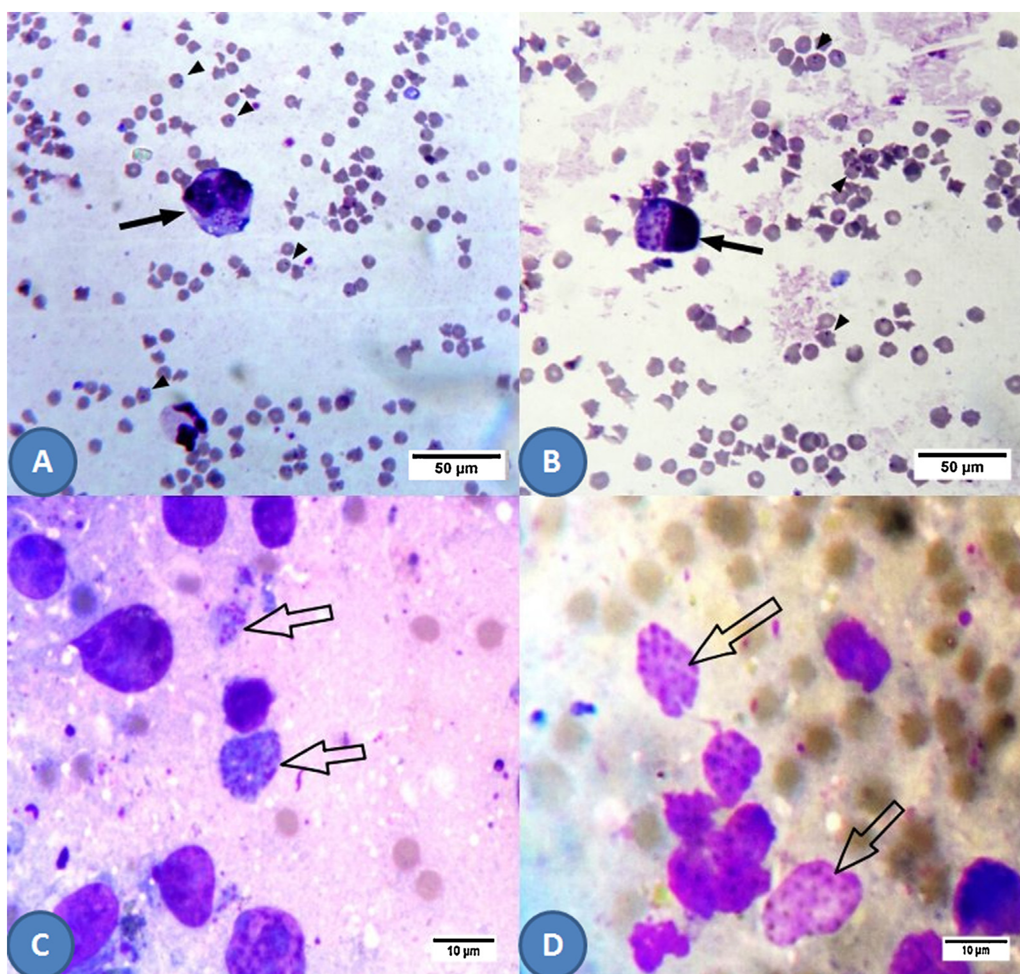

Fig. 1. The smears of peripheral blood (A and B) showing *Theileria lestoquardi* piroplasms (▲) in the red blood cells and Koch blue body in the lymphocytes (➡), and the impression smears (C and D) showing the ewe liver and the respective fetus spleen (⇨); Giemsa stain. Ewe (B and C); fetus (A and D).

the most obvious clinical signs in animals in the current study. The icteric mucosa, the presence of ticks on the animal body, submandibular and subscapular lymph node enlargement, weakness, increased respiration, and pulse rates were also observed. The packed cell volume (13%), red blood cell (RBC) concentration ($4.41 \times 10^3/\mu l$), and hemoglobin (3.9 g/dl) of ewe were less than the lower point of the normal range. Blood smears were prepared using blood taken from jugular vein, left to dry and fixed with methanol, and finally stained with the conventional Giemsa stain (Baharafshan, Iran). Direct impression smears were prepared from the dead ewe liver and aborted fetus spleen, followed by staining procedures with the Giemsa stain. Small pieces (1 cm × 1 cm × 1 cm) of liver and spleen were cut and stored at −70 °C for subsequent DNA analysis. The DNA was extracted from the liver and spleen using DNA extraction kit (Roche, Germany) and the extracted DNA was stored at −20 °C until subsequent analysis. Briefly, pieces from each infected organ were cut (3 mm × 3 mm; weight ≈25 mg) and lysed in 100-µl buffer followed by degradation with 10-µl proteinase K for 30 min at 55 °C. After addition of 270-µl binding buffer, the mixture was incubated for 10 min at 70 °C before adding 320 µl of absolute ethanol. The solution was briefly vortexed before transferring on to the column. The column was first centrifuged and washed twice with 500-µl washing buffer. Finally, DNA was eluted from the carrier with elution buffer.

3. PCR amplification

In order to amplify the part of 18S rRNA gene from the *T. lestoquardi*, primer sense (5′-CACAGGGAGGTAGTGACAAG) and antisense (5′-CTAAGAATTTCACCTCTGACA) were used (Zaeemi et al., 2011). The PCR was performed in final volumes of 100 µl containing 100 ng of DNA template, 10 mM Tris–HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 200 µM (each) deoxynucleotide triphosphates (dNTPs), 0.4 µM of each primer, and 2.5 U of *Taq* DNA polymerase (Invitrogen, Karlsruhe, Germany). The Bio-Rad Thermocycler was programmed as follows: initial denaturation (95 °C, 2 min) followed by PCR amplification with 35 cycles of 94 °C for 45 s, 56 °C for 45 s, 72 °C for 45 s, and a final extension of

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